

# The Artificial Insemination of Farm Animals

# The Artificial Insemination of Farm Animals

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Edited by  
ENOS J. PERRY

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THIRD REVISED EDITION

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# Preface

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Since 1945, when the first edition of *The Artificial Insemination of Farm Animals* was published, the volume of data resulting from research in artificial insemination has increased steadily, as has the practical use of the techniques developed by this research. Both in the United States and abroad, artificial breeding organizations have expanded their programs enormously.

The authors of this third edition have attempted to select from many experimental findings those new facts which will be of most practical help to livestock breeders, managers and technicians of artificial breeding organizations, veterinarians, students, teachers, and research workers. No one person could have chosen from the vast quantity of available materials the most important information on the several breeds of livestock, the problems of semen analysis and preservation, control of disease, and breeding and management practices. The editor is therefore highly gratified in having obtained the collaboration of leading authorities in the respective fields.

Artificial breeding is still practiced more with cattle than with any other animal, but in the last decade enormous advances have been made with poultry and swine. The chapters on these animals, as well as the chapters on disease and on the shipping of semen, have been either completely rewritten or extensively revised. The chapters on water buffaloes, frozen semen, and the evaluation of semen by chemical analysis are entirely new additions.

The chapter, "Advantages and Limitations," contained in the



previous editions, has been eliminated, because today nearly everyone who has an interest in "A I" is aware of its strength and weakness. They know that this method of breeding allows the livestock owner to utilize the germ plasma of the best proved sires for an extraordinary number of females, and that conception can often be effected in cases where natural mating is impossible or hazardous. With the advances in the preservation, freezing and shipping of semen, the inheritance of superior animals can be passed on to herds thousands of miles away. No herd owner today need go to the trouble and expense of owning his own sires.

The big challenge to the artificial breeder today is to select his sires only on the basis of superior inheritance and proved merit, to keep a constant check on their health, and to maintain conditions so sanitary that there is no possibility of transmitting genital diseases through infected semen.

On behalf of the co-authors and himself, the editor thanks the many friends who have made available reports and suggestions based on long and successful experience in the field of artificial insemination. Without the assistance so generously given, this book would not have been possible.

ENOS J. PENNY

New Brunswick New Jersey  
January 1960

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# The Artificial Insemination of Farm Animals

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# Historical

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ENOS J PERRY

In 1677 Anton van Leeuwenhoek and his pupil, Johan Hamm, discovered sperm by the use of a magnifying lens. As reported by Dobell (1932), they referred to the innumerable minute bodies as "animalcules" with the power of active forward motion,

The first scientific research in artificial insemination of domestic animals was conducted by the Italian physiologist L. Spallanzani, in 1780. He had attained success with several amphibious animals when he decided to experiment on viviparous species, using the dog first. Dogs were confined in Spallanzani's house, and after a lapse of 20 days the bitch manifested obvious signs of being in heat. Then, with semen at body temperature she was artificially inseminated, the semen being deposited directly into the uterus with a pointed syringe. Sixty-two days following insemination, the bitch gave birth to three pups, all of which resembled not only the mother but also the dog from which the semen had been taken. In 1782 Spallanzani's experiment was successfully repeated by P. Rossi and checked by Professor Branchi. These experiments proved the feasibility of inducing pregnancy by artificial insemination, with the resultant birth of normal offspring.

Spallanzani further discovered that the fertilizing power of semen resided in the sperm carried by the spermatie fluid. When the semen was filtered, the liquid that passed through was sterile, but the residue on the filter was high in fertilizing capacity. Spallanzani (1803)

also contributed to the knowledge of the effect of cooling on the prolonging of sperm life. He observed that freezing stallion semen in snow or winter cold did not necessarily kill the "spermatic vermiculi," but held them in a motionless state until exposed to heat, after which they continued to move for seven and a half hours.

His discoveries gave rise to intensive investigations of the sex cells and the physiology of fertilization, but these studies did not, as one might have expected, stimulate any further developments in artificial insemination for a long time. In fact, it was not until the latter part of the nineteenth century that new experiments were undertaken both in Europe and America. Concerning research, W. Heape (1897), of England, reported that a dog breeder, Everett Millais, between 1884 and 1896 artificially inseminated a total of 19 bitches, of which 15 produced young. Heape wrote that in the light of this work it appears that artificial insemination is easy, that conception is as readily induced in that way as by normal coitus, and that a single ejaculation serves several bitches. He claimed that the method could be used to cross dog breeds whose natural mating is impossible because of difference in size, and he suggested the plan as a means of studying genetic and teleogenic factors. Heape also referred to research with mares, in which the object was to overcome sterility. Pearson, professor of veterinary medicine at the University of Pennsylvania, wrote to Heape, stating that he and some other veterinarians had been successful in the artificial insemination of mares on a number of farms.

In Europe, Plonnis had artificially inseminated a bitch in 1876, an undertaking repeated by Albrecht in 1894 to investigate problems in telegency, which at that time held the undivided attention of breeders of domestic animals. Artificial insemination was first used in horse breeding in Europe in 1890, when the French veterinarian Repiquet advised its use as a means of overcoming sterility. In the stud farms of several European countries the percentage of conceptions obtained was very low, so investigations for the purpose of improving this condition were then begun. Professor Hoffman of Stuttgart recommended the use of supplementary artificial insemination following natural mating. He wrote "In any rationally conducted horse breeding attempt it is necessary, as soon after copulation as possible, to introduce the sperm present directly into the uterus through the uterus orifice." He gave a detailed description

of his techniques and the instruments necessary. After the stallion covered the mare, the semen deposited in the vagina was collected from a depression in the lower vaginal wall with the aid of a speculum and spoon. The semen was then sucked up with a special syringe, diluted with cow's milk, and introduced into the uterus. He contended that the transference of semen to other mares was not a matter of practical importance, and left studies in that field to others. In Denmark during this same period Sand and Stribolt obtained four successful conceptions after artificially inseminating eight mares. Reporting at the Northern Livestock Conference in Copenhagen in 1902, Sand said that the most important feature of the practice was the economical use of the semen of a valuable stallion. Thus, whereas the others had viewed the method as a measure to solve the problem of sterility, Sand considered its potentiality for the widespread improvement of farm animals.

It was in Russia, however, that the method was first taken up seriously as a means of attaining this new objective. The best known Russian investigator, and a leading pioneer in artificial insemination, was E. I. Ivanoff (1922). In 1899 he was requested by the chief of the Royal Russian Stud to determine the possibilities of the method for use in horse breeding. Under his direction, artificial insemination was practiced by numerous stud farms, but the results were not uniformly good. He noted, however, that where he did the work or where it was done under his oversight, the conception rate was somewhat higher than that obtained by natural mating. At Askaniya-Nova in 1912 the insemination of 39 mares resulted in 31 conceptions, whereas there were 10 conceptions from the natural breeding of 23 mares. He also succeeded in inseminating birds.

Ivanoff was the first to undertake successfully the artificial insemination of cattle and sheep. While working with the stud farms, he asked permission of the Ministry of Agriculture to institute experiments on these species. The minister referred him to the Agricultural College at Moscow so that the investigations could be conducted on the property of the college. However, a committee of the professors objected to having such experiments tried on their cows. Ten cows were bought, and Ivanoff obtained successful results with some of them. He had similar success with sheep at the livestock farm and station at Askaniya-Nova. His results awakened much interest, and a physiological section was established in the veterinary

Laboratory of the Ministry of Agriculture, with the special objectives of studying the physiology of fertilization and training veterinarians in the techniques of artificial insemination Ivanoff headed the section, and during the years prior to World War I between 300 and 400 men were trained and sent out as practitioners of artificial insemination. A very considerable increase in the number of animals mechanically bred resulted. Between 1913 and 1917, a total of 323 mares were artificially inseminated in Japan.

Beginning in 1914, G. Amantea, professor of human physiology at the University of Rome, began experiments in spermatology, using the dog, cock, and pigeon (Bonadonna, 1937). He is reputed to have devised the first artificial vagina, the one here pictured (Figure 1). It was used to collect semen from the dog. A little later the Russian investigators Milovanov, Filippov, Kuznetsov, Neumann, Schivnova, Skatkin, and others utilized the reports of Professor Amantea as an aid in their development of artificial vaginas suitable

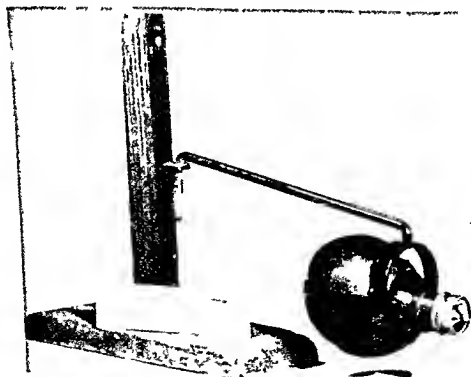


Figure 1 The earliest artificial vagina invented by Professor Giuseppe Amantea of the University of Rome was used for the collection of dog semen.



for the bull, stallion, and ram, and thereby expedited the artificial insemination program in its initial stages (Milovanov, 1938). The studies of the physiology of the sperm by these men were also a valuable contribution to the science of livestock breeding.

One of the early English workers was Arthur Walton (1933), who reported much useful information pertaining to the handling of semen, based on many trials. Walton pioneered with Prawochenski (1936) in long-distance shipments. In 1936, sperm was collected from a Suffolk ram at Cambridge, cooled to 10° C., transferred to a thermos flask containing chipped ice, and airmailed to the Pulawy Zootechnical Institute in Poland. Five cures were inseminated with semen two days and three hours old. Two ewes became pregnant, and one gave birth to a ram lamb that plainly possessed Suffolk characteristics.

By 1938 the number of animals inseminated in Russia had risen to 120,000 mares, 1,200,000 cattle, and 15,000,000 sheep. In sheep farming artificial insemination had become very popular, and in many studs and flocks it was the sole method of breeding.

Eduard Sorensen (1938) of the Royal Agricultural and Veterinary College, Copenhagen, a close student of the Russian technique, and Jens Gylling-Holm, agricultural adviser of Tranebjaerg, organized the first cooperative artificial breeding association in Denmark in 1936. It had 220 members, and 1,070 cows were inseminated the first year. The average number of inseminations per pregnancy was 1.68, a rate slightly better than the natural service in the same herds. Fifty-nine per cent conceived on first insemination.

The first demonstration in America proving that large numbers of cattle could be successfully bred by artificial insemination was conducted at Pabst Farm, Wisconsin. Howard Clapp, the manager, reported on May 14, 1938, that from 1934 to that time 130 calves had been born on the farm as the result of artificial insemination (Clapp, 1938).

The first cattle breeding organization to use artificial insemination in the United States began operations in New Jersey on May 17, 1938, as Cooperative Artificial Breeding Association No. 1 (Perry and Bartlett, 1955). It started with 102 members, and it was modeled after the Danish system; 1,050 cows were enrolled, and it grew rapidly. The first technician was J. A. Henderson of Canada, and his associate and advisor for two months was A. F. Larsen of Denmark, who had successfully performed the work in his country's first

association The number of dairy cows inseminated by the 56 cattle breeding studs of the United States in 1961 totaled 7,047,148 from 863,781 herds—about 36.7 per cent of the milk cow population of the country One bull has been bred to more than 140,000 cows, and many others to from 30,000 to 50,000—fantastic figures to dairymen a few years ago Two artificial insemination bulls each have over 1,200 production tested daughters By the end of 1958, several other countries had made still more amazing strides in increasing the percentage of cows being bred artificially These were Denmark, 98 per cent, Japan, 92 per cent (dairy), Netherlands, 65 per cent, England and Wales, 58 per cent, and Sweden, 26 per cent Marked headway was reported for Ireland, with 26 per cent, West Germany, 25 per cent, and France, 23 per cent In a world survey conducted at the request of the National Association of Artificial Breeders of the United States, reports from 54 countries revealed that a total of 22,486,101 cows and water buffaloes were artificially inseminated in 1958

With the newer knowledge of preserving and shipping bull semen, a system of centralized operation was developed for the cattle improvement program throughout much of the world The keeping of fewer but better sires at a central point from which many affiliated groups can be served is effecting tremendous economies for the breeding organizations

A new landmark in the field of artificial insemination was established in 1949, when C Polge, A U Smith, and A S Parkes (1949) discovered a practical method for the long time preservation of the semen of certain species by deep freezing to temperatures of  $-79^{\circ}\text{C}$ , by means of dry ice ( $\text{CO}_2$ ) Because glycerolized diluter protected fowl sperm markedly against low temperatures, Polge and L E A Rowson (1952) tested the fertilizing capacity of bull sperm that had been buffered with egg yolk sodium citrate and equilibrated with glycerol diluter for several hours before freezing The resulting conception rate was satisfactory The long distance shipping of ampoules of frozen bull semen within and between some countries has become a common practice The refrigerants are dry ice or liquid nitrogen The latter was made practical by the American Breeders Service of Madison, Wisconsin (1957) With the aid of electric power or the liquid nitrogen some stores of semen have been kept at temperatures much lower than that of dry ice The Waterloo Cattle

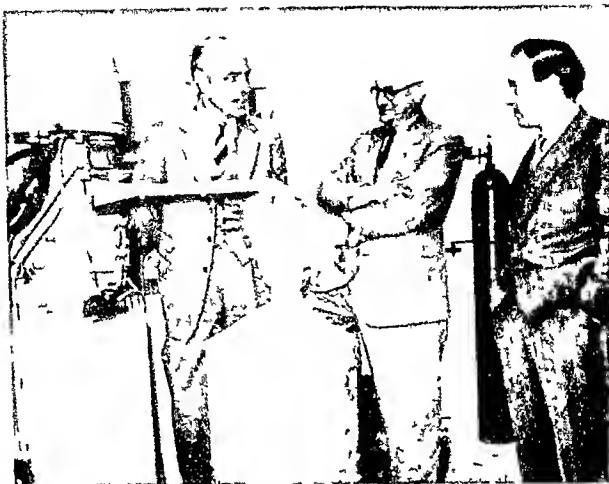


Figure 2 Dr Joseph Edwards Dr A S Parkes and Dr C Polge of England, who were associated with the discovery of the technique now used for freezing sperm at very low temperatures

Breeding Association of Waterloo, Canada, was the first breeding organization in the world to operate a 100 per cent frozen semen program, beginning in December 1954. The Ontario Veterinary College at Guelph, Canada, conducted early trials in the large scale processing of frozen semen and the developing of practical equipment for storage and field use.

Artificial insemination has been used experimentally to yield hybrids or bastards between species that do not voluntarily mate. For example, zebroids have been produced by crosses between the male zebra and mares, and progeny have resulted from crossing domestic cattle with the zebu and hison. On the other hand it was proved impossible to cross the sheep and goat, the dog and fox, and the European rabbit and hare.

The number of workers in the field of artificial insemination has increased rapidly, and much literature has accumulated upon the subject. Improved techniques have been developed for the collection

and handling of semen of the common farm animals, and for their insemination. Along with these advances many new facts have been established concerning the biology and biochemistry of sperm, of the secretions of the reproductive glands of the male, and of ovulation and heat and their related phenomena in the female. Opportunities for further studies and discoveries are unlimited.

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# Male and Female Organs of Reproduction

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JOSEPH EDWARDS

An understanding of the structure and functioning of the organs of reproduction is essential if an intelligent application of the technique of artificial insemination is to be made. The subject can be dealt with only very briefly in the present chapter.

## THE MALE ORGANS

*Structure.* The organs of reproduction in all domesticated species of animals are essentially similar; a description of them as they occur in the bull will be given. They consist of the testes or testicles (see Figure 3), which are suspended outside the body cavity in the scrotum. The spermatozoa or sperm are formed inside these testes. A coiled tube leads off from each testis and forms a body, the epididymis, in which the sperm mature and are stored until ejaculation occurs. From this a tube passes through the inguinal canal into the body cavity. These tubes come together and enter the urethra, a structure which is continued within the erectile penis. The point at which the tubes make contact with the urethra is close to that at which the bladder also opens, and from this area both semen (during mating) and urine (during micturition) pass to the exterior.

Also in this area are to be found accessory glands, the largest of

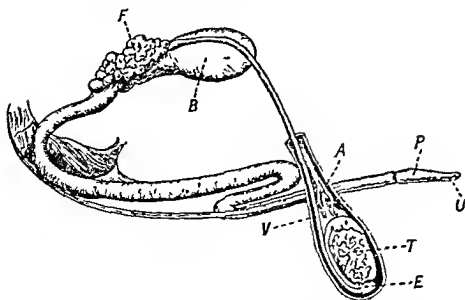


Figure 3 Reproductive organs of a bull A Blood vessels in scrotal cord supplying testis T Testis—forms sperm E Epididymis—stores sperm V Vas deferens—conducts sperm in ejaculation F Vesicula seminalis—supplies fluids diluting spermatozoa on ejaculation P Penis—intromittent organ U Opening of urethra—conducts semen and also urine from bladder B Bladder—stores urine

which are the vesiculæ seminales (F in Figure 3) Others include the prostate and Cowper's glands During copulation the sperm from the testes are diluted, to an extent which varies with the species, with liquid secretions from these glands, and the resulting mixture is called the semen In the boar the cells lining the urethra also contribute materially to the volume of the semen

The surface of the penis is well supplied with numerous sensory end organs, and the fact that the organ is extremely sensitive should always be borne in mind It is sensitive to manipulation (the sheath and not the penis should be handled at the time of semen collection), and it is sensitive to temperature If the inner surface of the artificial vagina is too hot, pain will be experienced, if it is too cold—in some cases as little as 2° F below blood heat—the ejaculatory process will not be completed The penis is sensitive also to the surface of the inner lining of the vagina A high quality rubber with a perfectly smooth surface should always be used

**Functioning of Testes** The testes have two important and quite distinct functions—the production of sperm and the production of

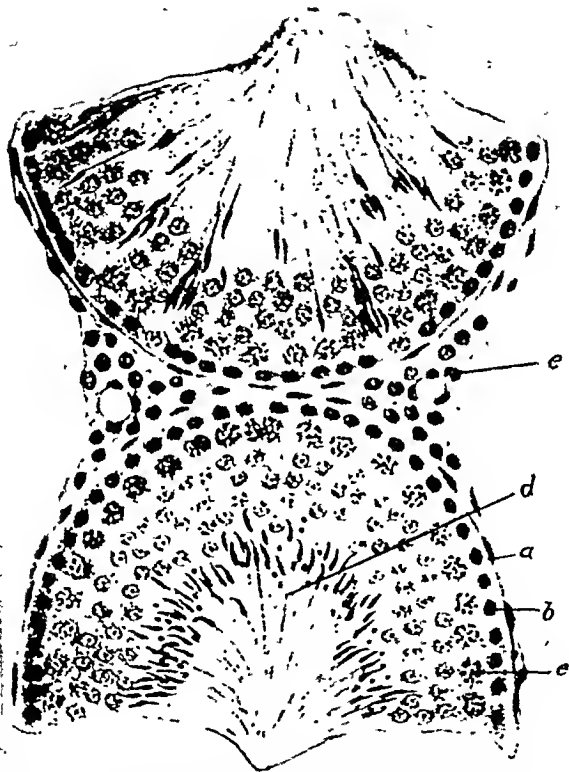


Figure 4. Section through seminiferous tubules of the testis a. Basement membrane. b. Spermatogonium c. Spermatocyte d. Sperm in cavity of tubule e. Interstitial tissue containing blood vessels (F. H. A. Marshall, *The Physiology of Reproduction*, London, 1900)

the male sex hormone. To understand the way in which these functions are performed, it is necessary to make a most intimate study of the organ. The greater part of each testis is composed of minute tubes, which under the microscope are seen to have a cross section appearance, as shown in Figure 4 (one half of each of two tubes is to be seen). The sperm function is carried out within the tubes, and the male sex hormone is produced by the tissue between the tubes. Between the base of each tube and its cavity in the center a progressive series of changes in cell structure take place, and the original "mother cells" at the base give rise in the end to the sperm. During the process there is a reduction by half in the number of chromosomes that each cell normally contains and sperm capable of determining the male or female sex after fertilization are produced in equal numbers. All efforts to control sex by attempting to segregate, destroy, or increase the survival power of one or the other type of sperm so far have failed to produce results.

**Onset of Spermatogenesis** There are records of bulls being proved fertile, by natural service, when as young as six months and at establishments where bulls are reared from calfhood for artificial breeding units semen of apparently good quality has been collected from this age onwards. Breed, individuality, and growth for age are factors still to be examined separately and together, and it will also be interesting to know the effect upon growth and sperm production later in life of a period of activity shortly after puberty which is followed by one of inactivity. Frozen and banked collections of semen from a very young sire offer the prospect of planned progeny test results with a saving of nine to twelve months' waiting time.

**Rate of Sperm Production** The rate at which sperm production (spermatogenesis) occurs is of fundamental importance in artificial insemination work. Individual males undoubtedly inherit varying rates of testicular activity, just as females vary in inherited levels of fertility or milk secretory activity. A wide range in rate of sperm production may go unnoticed in the case of sires used by normal mating in herds of average size, but important differences are revealed when sires are subjected to regular collection routines.

A standard for the rate of sperm production has been worked out for the rabbit; it is found that 1 gm. of testis produces 100 million sperm per week (see Figure 5). This is equal to nearly 5 million sperm per hour, or 80,000 per minute, for a rabbit with testes weigh-



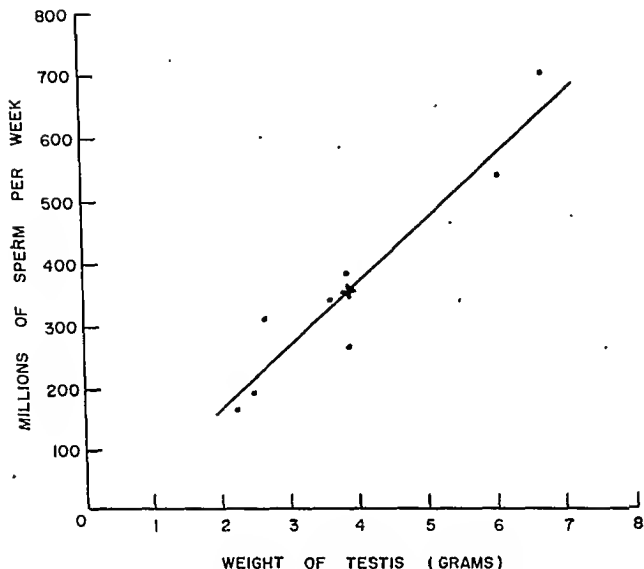


Figure 3. Rate of sperm production in the rabbit; comparison between weight of testis and number of sperm per week.

ing 8 gm. Standards for rate of sperm production for bull, stallion, ram, and hoar have yet to be defined in this way, although for the bull recent American research suggests that it may not fall far short of that for the rabbit.

One or two sample ejaculates are unlikely to provide much evidence of differing rates; because of storage capacity, several ejaculates have to be collected over a short period of time if different levels are to be revealed. In an experiment with a group of 15 bulls, from each of which ten ejaculations were collected in a space of two hours, it was found that the bulls did not arrange themselves in a stable order of merit for sperm production until after the fifth or sixth collection. In Table I the total number of sperm in the first two collections and in ten collections are given for five of the bulls.

TABLE I

Bull	Total sperm in 2 ejaculates (millions)	Total sperm in 10 ejaculates (millions)
1	6 584	28 544
2	7 469	20 303
3	9 647	18 961
4	8 236	15 959
5	4 089	11 036

Although quite a lot is known about internal factors affecting the onset of sperm production—the effect of the pituitary gland for example—there is still little evidence to show the way in which the rate may be influenced. Further research will no doubt yield results in this field. The effect of the pituitary gland hormone in the male is qualitative but not quantitative that is the presence of the hormone is essential for spermatogenesis but larger amounts of it will not increase the numbers produced. This contrasts with the behavior of the comparable gland in the female—if one ovary is removed the remaining ovary will compensate by producing twice its normal number of eggs. If one testis is removed in the male the remaining testis will not compensate but will continue to produce at its own characteristic level.

Temporary infertility may be observed in males when there is a sudden change from a high plane of nutrition to one which is normal or low. The first state of affairs—high to normal—frequently arises in the case of males produced in a very high state of condition for pedigree sales and subsequently brought down to normal feeding in a commercial unit. The effect sets itself right in a short time (one or two months). The successful treatment of bulls of low fertility with ascorbic acid has been reported but the evidence is not conclusive.

In temperate climates there is as yet very little evidence of seasonal variations in the number or quality of sperm produced in the bull. The possibility of such variation in warmer climates is not ruled out and in the case of one breed of sheep (Shropshire) a deterioration in quality of semen in hot summer months has been described.

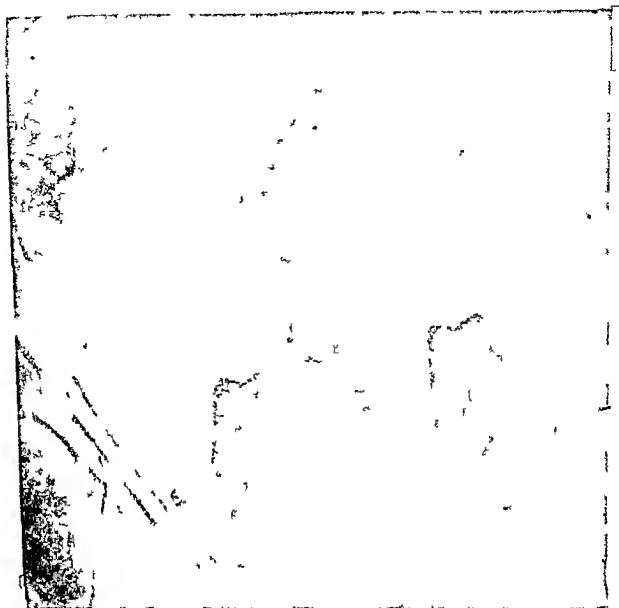


Figure 6 Living bull spermatozoa Ultraviolet photomicrograph  $\times 4243$  (J. L. Hancock Jour. Expt. Biol. XXIX [1952] 445)

The sperm are very sensitive to high temperatures, and the scrotum is a mechanism for regulating temperature. Low fertility is liable to occur after a period of fever in the animal.

**Flow of Sperm in the Male Tract** The flow of sperm in the male tract is extremely interesting and significant. There is, of course, a constant flow from each testis, and the sperm leave from the top end of the organ as it hangs in the scrotum. A tube runs down the side of the testis to its base and here coils extensively to form the main part of the epididymis (E in Figure 3). This structure can be felt through the scrotum—in the bull it is about the size of a walnut—and its condition is a very crude indicator of fertility. In a fertile

male it is firm to the touch, whereas it is flaccid in one of poor fertility. The sperm are stored in this part of the epididymis, and it is believed that here they undergo a maturing process. At the time of ejaculation the sperm are moved by rhythmic contractions through the tube which connects the testes with the urethra, where, as already described, they are diluted with secretions from the accessory glands to form the semen.

In the case of a male which is not given service the sperm continue to be produced at a normal rate. The flow is not interrupted, and the old sperm are moved out of the epididymis into the tube (vas deferens) by the arrival of the new. The evidence suggests that, masturbation excepted, the only way in which the old sperm are disposed of is by disintegration and absorption by the walls of the tube. It is clear therefore that the sperm content of the epididymis at any time is a blend of sperm of different ages, and that it is necessary to study the rate at which individual bulls are capable of producing sperm so that a schedule of collection may be arranged which will give the best type of ejaculate. Infrequent collections will result, in the case of a bull of high producing ability, in initial ejaculates of aged or dead sperm. A case is known of a bull from which, if the collection interval is as long as ten days, three to four ejaculates of such semen have to be discarded before one of good quality is obtained. On the other hand, overfrequent collection may result in the ejaculation of immature sperm with less than normal ability to fertilize and sustain storage conditions.

**Semen Characteristics.** Species differences in semen characteristics exist chiefly in volume and density of sperm, and these in turn cause differences in survival (storage) ability and the number of females which may be inseminated from one ejaculate. Average values for the bull, ram, stallion, and boar are given in Table II.

It will be seen that the large volume of semen produced by the stallion and the boar neither stores well nor is capable of being used for very many females. In these two species the sperm at the time of ejaculation are diluted to a great extent by the secretions from the accessory glands, and storage times and inseminating capacities more comparable with those of the bull and the ram await the discovery of new methods. The life of the sperm is limited by the sources of energy it contains—after leaving the male it is incapable of using external sources. The storage process aims at reducing the

TABLE II

Animal	Average volume of ejaculate (c.c.)	Average concentration (millions per c.c.)	Storage survival time* with retention of fertilizing capacity (days)	Number of females per ejaculate
Bull	4	300-2,000	4-6	100-600
Ram	1	800-4,000	5-7	40-100
Stallion	50-100	30-800	1-2	8-12
Boar	200-250	25-1,000	$\frac{1}{4}$	4-8
Cock	0.2-2.0	$\frac{1}{2}$ -60	Always use fresh	6-10

\* For liquid (non-deep freeze) state.

expenditure of this limited amount of energy by controlling sperm activity, and this is effected in the case of dense concentrations (for example, bull and ram) both by the accumulation of carbon dioxide gas produced as an end-product of sperm metabolism and by cooling the semen to a temperature of 5° C. Before the time when egg yolk and milk came to be used in the artificial dilution medium, it was necessary to cool slowly; but the use of these substances, acting in a way still undefined, makes possible the simple cooling technique now practiced. In the case of the very dilute boar and stallion ejaculates it can readily be imagined that the sperm will have used up a considerable part of their energy before either carbon dioxide concentration or cooling is able to control their activity. There may be additional factors at work, but it is thought that the dilution itself has a considerable negative effect on storage capacity in these two species.

The individual sperm (or spermatozoon) is an extremely small structure somewhat less than  $\frac{1}{500}$  of an inch in length and tadpole-like in shape (see Figure 7). Inside the head is the nucleus which contains the chromosomes—rod-like structures on which the units of heredity (genes) are located. The tail is used, as in the tadpole, as a means of locomotion. The swimming action of the sperm takes place inside the female tract in the liquid medium that bathes its surface. The egg (or eggs) produced by the female may be penetrated by more than one sperm, but one sperm only makes contact

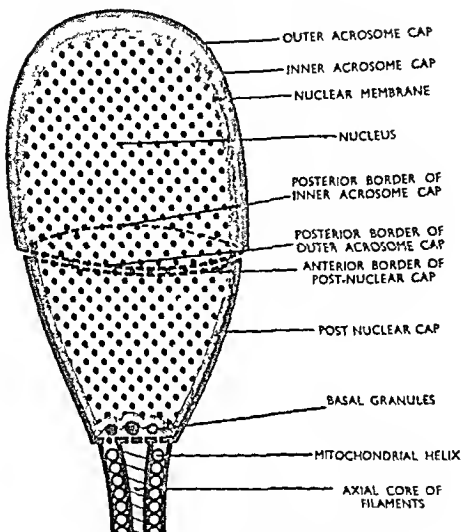


Figure 7: Probable structure of the head of bull spermatozoa (W H Bishop and C' R Austin Endeavour XVI (1957) 148)

with the nucleus of the egg. The fusion of the nuclei of sperm and egg constitutes fertilization (see Figure 8)

It is as yet impossible to define the minimum number of sperm required for fertilization by artificial insemination. It is obvious that there is considerable waste in normal mating wherein 4,000 to 5,000 million sperm are introduced into the female. For a time it was thought that the lower safety limit was 250 million sperm per insemination, but more recent work suggests that about 10 to 15 million will suffice when the intracervical or intrauterine methods of in-



Figure 8. *Hydraides hexagonus* (Annelida): Spermatozoon entering an egg. Head (nucleus), two middle pieces, part of the flagellum, and a very small part of the acrosome are included. In this species it is considered that lytic material comes from the spermatozoon and erodes a hole (clear area) in the egg membrane. The large sperm head can then pass into the egg. Approximately  $\times 19,385$ . (Courtesy Colwin and Colwin, Queens College.)

semination are used. (This is equivalent to 1 e.c. of a 1:50 dilution of an ejaculate of 800 million density.) In the future this number may be still further reduced, but it must be remembered that the sperm encounter many hazards during their ascent in the female tract and that large numbers fail to reach the site at which fertiliza-

## Artificial Insemination of Farm Animals



Fig. 9 Head of bull spermatozoon showing an abnormal acrosome characteristic of the spermatozoa of certain sterile bulls. Electron micrograph, gold-palladium shadowed  $\times 3960$  (U. L. Hancock Jour. Expt. Biol. XXX [1953] 50)

tion takes place. The intracervical and intrauterine methods of insemination overcome some of these hazards.

It must be emphasized that the spermatozoon is an extremely delicate organism and that it requires most careful treatment at each stage of the artificial insemination process. Substances harmful to it include disinfectants possessing bactericidal properties—but not antibiotics—and metal surfaces. In the former case sperm should not come in contact with equipment cleaned with disinfectants, nor should it be exposed in an atmosphere containing them. It is obvious that neither metal catheters nor metal tubes should be used to hold sperm.



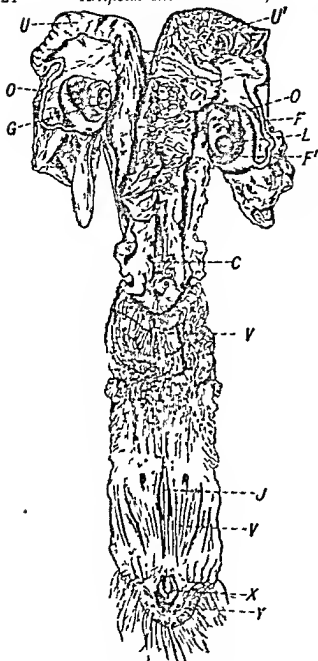
**Sex Drive.** It was noted earlier that the testes exercised a hormone function in addition to their sperm function. The male sex hormone produced by the testes is responsible for the development of the secondary male characters—for example, the thickening of the crest over the withers, the strong horn, the deepening of the vocal sounds in the bull, and the sex drive or willingness to serve. This last function is quite unrelated to that of sperm production, a fact that is obvious from examples of males which are keen at service although sterile, and from contrary cases. The presence of the hormone is the foundation of "maleness," and the mating process becomes essentially a reflex act governed by the nervous system.

The behavior of males prior to and during service constitutes a fascinating aspect of animal behavior. In certain species of birds and animals the display on the part of the male that precedes the act is elaborate and is carried out according to a definite pattern. It may not appear to be either as elaborate or as definite in the case of bulls, but sufficient is known for it to be realized that the mechanism is delicately balanced and dependent, especially in the case of older animals, on what the psychologist would describe as conditioning. In this respect the bull is very much a creature of his environment—he becomes used to his surroundings, the personnel who look after him, the preparations for semen collection, and the technique itself. A sudden change of any one of these factors may upset him. If, on the other hand, a bull becomes slow at service, it is sometimes possible to stimulate him by creating a sense of frustration, either by allowing him to see other bulls serve before he is permitted to do so, or by leading him up to the cow as if he were going to be used but immediately returning him to his stall. The Russian workers who have made a close study of bull psychology recommend that this latter course should be followed daily with inactive bulls.

#### THE FEMALE ORGANS

**Structure.** The structure of the female organs of reproduction is similar in our domesticated species of livestock, and once again that of the bovine will be described (see Figure 10).

The ovaries, which give rise to the eggs or ova, are two in number and are connected to the back of the female inside the main body cavity. The uterus, or womb, is not in direct contact with the



- O Ovaries — produce Graafian follicles containing ova
- G Graafian follicle — contains ovum (egg)
- L Corpus luteum — formed from ruptured follicle
- F End of Fallopian tube or oviduct — catches ovum from ruptured follicle
- F Fallopian tube — conducts fertilized ovum
- U Uterine cornu or horn — nourishes developing ovum (embryo)
- U' Uterine horn cut open — nourishes developing ovum (embryo)
- C Cervix — shuts off developing embryo from exterior
- V Vagina
- J Opening of urethra — exit for urine
- X Clitoris — erectile and sensory organ
- Y Vulva — at external opening

Figure 10 Reproductive organs of a cow Part of canal cut open

ovaries, but the passage of eggs into it is effected through two oviducts (Fallopian tubes), which protrude from each horn of the uterus and end in funnel-shaped openings. These funnels, particularly around the time of ovulation surround the ovaries so that the

eggs do not fall into the body cavity but enter the uterus. The egg, though minute, is much larger than the sperm and is about  $\frac{1}{180}$  inch in diameter.

The uterus proper consists of two horns and a body. The surfaces of these structures in the ruminants (cow, sheep, goat, and so on) are found to possess a large number of processes called cotyledons, which become much enlarged during pregnancy; and it is to these that similar structures developed around the embryo attach themselves, and through them that the developing young is nourished. These surfaces are also richly supplied with glands which produce a fluid secretion; it is through this medium that the sperm ascend to fertilize the egg.

The uterus ends in a firm canal called the cervix, and from this point connection with the exterior is made by the vagina and vulva, or external opening. The state of the cervix, the opening into the womb, varies both with the species and with the stage of the reproductive cycle. In the case of the normal cow the cervix opens slightly during estrus (or heat), so that at this time it is possible to pass the inseminating tube through into the uterus. Tubes of a narrower diameter may have to be used for heifers and the smaller breeds of cow. In normal mating the penis of the bull does not penetrate the cervix, and the semen is deposited in the vagina close to the entrance. In the ewe it is not possible to do more than introduce the semen into the entrance of the cervix. In the mare, on the other hand, the dilation of the cervix during estrus is considerable; and to judge from the difficulty experienced in withdrawing semen from the vagina after a normal service, it is concluded that the bulk of it is deposited in the uterus during the act.

Between two heat periods the opening of the cervix contracts; and if pregnancy follows service or insemination, it becomes sealed off by a secretion which is sticky and gelatinous.

*Functioning of the Ovaries.* When puberty or sexual maturity is reached, the ovaries begin to function with a rhythm or cycle of activity, the most evident manifestation of which is estrus or heat. The duration of heat is about 19 hours, and the interval between heats  $19\frac{1}{2}$  to 20 days. There are individual and seasonal variations in these times.

As in the case of the testis, an understanding of the functioning of the ovary calls for a more detailed study of its structure. The tissue

in the surface layer area contains very large numbers of potential egg forming cells. There are many more of these than are required for the breeding life of the female—the estimate for the heifer is 75,000. As the heat period is approached one or a few ova begin to mature, each in a small follicle or sac. The follicle grows, and at the onset of heat is grape like both in appearance and size. Its outer wall encloses chiefly the mature egg and a quantity of liquid. About 14 hours after heat is over in the cow—about 24 hours before it finishes in the mare and at about the end of heat in the ewe and sow—the wall of the follicle ruptures and the liquid content oozes out, washing the egg into the funnel shaped ending of the tube leading to the uterus. Fertilization in the presence of sperm takes place in this tube, and the egg then passes into the uterus. The various stages in the estrous cycle here described are shown in Figure 11.

The life of both the egg and the sperm in the uterus is limited, and failure to achieve conception may result from wrong timing. This is particularly important in the mare, because in this species the duration of heat is always a matter of several days. Mares tend to have a characteristic duration of heat during the breeding season, and insemination planned to coincide with the last two to three days will give much better results than one early in the period.

It is now necessary to examine the change that occurs in the ovary after the egg has been shed. The inner surface of the follicle in which the egg matured now proceeds to produce a different kind of tissue, which comes rapidly to fill the cavity. This tissue forms, in the space of a few days, a solid structure known as the yellow body, or corpus luteum. If conception has taken place, this yellow body (the corpus luteum of pregnancy) persists almost to the end of gestation. Its removal from the ovary (see Figure 12) in the early stages will cause an abortion. The yellow body is responsible for a hormone which, among its other effects, prevents any further production of eggs or symptoms of heat.

If mating or insemination does not take place during heat or if conception fails to follow such mating, the yellow body normally reaches its maximum size and activity at about the ninth day after heat and thereafter proceeds to become smaller and inactive. During this phase of regression a phase of follicular and egg producing activity begins again in the same or other ovary, reaching its peak once more at the next heat period. Follicular activity is controlled quanti-

# Male and Female Organs of Reproduction

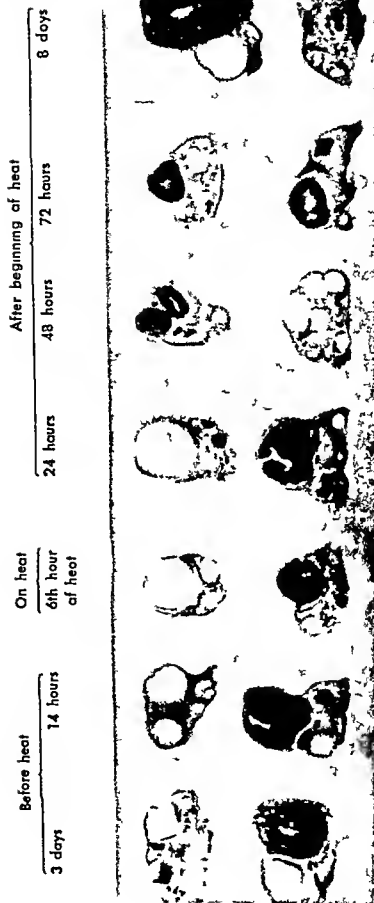


Figure 17 Ovaries of cows at different stages of the estrous cycle. The two ovaries of the animal are shown one above the other in the top row the ripening follicle is seen; it ruptures between 24 and 48 hours after the beginning of heat and forms the new corpus luteum which at first is dark from a blood clot and later in the process of reorganization becomes a paler body, in the meantime increasing in size. In the bottom row, which shows the ovary with the old corpus luteum of the previous heat period, the stages of its degeneration are seen (John Hammond, *Physiology of Reproduction* in the Cow, 1927. Reproduced by permission of the Cambridge University Press)

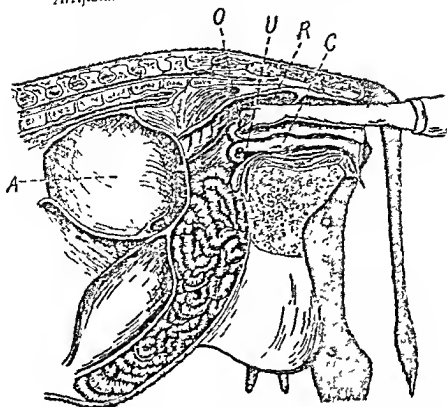


Figure 12 Position of reproductive organs in cow C Cervix. R. Rectum.  
U Uterus O Ovary.

tatively by a hormone (FSH) produced by the anterior pituitary gland (cf., Functioning of Testes), and the number of young to which the female gives birth depends very largely on the amount of this hormone that circulates in the blood. In the mare and the cow it is normally sufficient to cause the maturation of one egg at each heat period. In the sow, on the other hand, several eggs are ripened, and in the ewe, twinning, as a result of the production of two eggs, is common. The latter species provides evidence of the fact that the plane of nutrition affects fertility—ewes “flushed” with a protein-rich supplement before mating produce larger lamb-crops as a result of a higher rate of egg production. This rate may also be influenced by injection of the follicle-stimulating hormone now obtained from the blood of pony mares which are between the fiftieth and ninetieth days of pregnancy.

The use of this hormone to increase the lamb-crop from ewes and to get them to breed out of season has been tried successfully; and

a field experiment in cattle, associated with the routine functioning of an artificial breeding center, is being tried out in England for the first time in 1959. Although twin calves are not wanted in purebred dairy cattle breeding, it is quite different where beef calves are requested, either from beef-type cows or from commercial dairy cows for which "beef" semen is used. (In England and Wales semen of "beef" breeds is used for one-third of all artificial breeding matings.)

**Abnormal Ovarian Conditions.** The failure of cows and heifers to come into heat may considerably upset a herd breeding program. This phenomenon may result from a variety of conditions affecting the functioning of the ovary. A relatively rare cause is one in which the ovaries become completely inactive (anestrous); they are much smaller than normal in size and produce neither follicles nor yellow bodies. There is some evidence to suggest that this may be caused by a low plane of nutrition, which might particularly affect a group of young breeding heifers wintered under rough conditions. Improved nutrition prior to breeding may prevent this state of affairs; but if it exists either in a group of heifers or an individual cow, it may be overcome by the injection of the follicle-stimulating hormone (PMS, 2,000 international units). It is necessary by an examination per rectum (Figure 12) to be certain that the ovaries are in the anestrous state before using this treatment; for if "still heats" (that is, the egg being shed without signs of heat) are occurring and there is a corpus luteum in the ovary, there is the danger that two or more eggs may be shed.

In individual cases and for a reason not yet understood, the yellow body may persist in the ovary in the absence of conception. In this state the ovary cannot return to the follicular phase, and the yellow body has to be expelled (per rectum) before it may be resumed. In the majority of cases ovulation and heat occur two or four days after the operation. An ovary in which the follicle has become cystic and from which the egg is unable to be expelled may also be treated in this way. In the former case the symptom is an absence of heat, and in the latter this, too, or a prolongation of heat or a frequent series of estrous periods.

With regard to other types of infertility in cows, it is true to say that artificial insemination experience has had a very sobering effect on many theories that flourished before this new body of experience became available. Inseminators will be puzzled by good results in

herds in which the physical condition of the cows and even the state of the breeding passage leave much to be desired, and, on the other hand, by poor results in clean, well managed herds in which all factors appear to be normal. In general it is true to say that both under- and overfeeding are enemies of normal fertility, and the ideal state is one in which the animal is in a hard thriving condition. Further research alone can provide the answers to problems to which these explanations and generalizations do not apply.

In pregnancy in the cow the blastocyst stage lasts until about 18 days and the embryonic until about six and one half months, during which time the growth of fetal membranes is relatively greater than that of the embryo. In the last two months of pregnancy—the fetal stage—this is reversed, the fetus growing more rapidly.

It is in the first and the earlier part of the second stages of pregnancy that embryonic death occurs, and this is mainly responsible

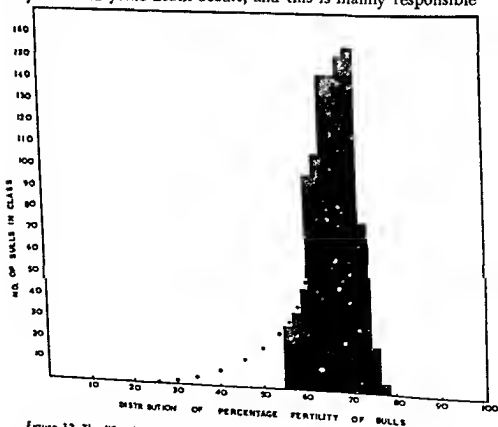


Figure 13 The "Fertility Barrier." The CR (Conception Rate) index (90 to 120 days) is never likely to go beyond 75 to 78 per cent in a normal cow population, due largely to embryonic death.



for the initial non-return rate (30 to 60-day) and the further decline of about 10 points that occurs up to the 90 to 120-day non-return index for the same group of females. The cause is unknown: One theory associates the phenomenon with a lack of a substance in the uterine secretions.

A practical result of this pregnancy defect is that a "population" of cows in a variety of farm conditions imposes a "fertility barrier" beyond which differences in male fertility cannot be demonstrated. Figure 13 shows, for more than 1,000 bulls with non-return rates (90 to 120-day) calculated for not fewer than 500 cows each that the barrier exists at around 78 per cent. Relative degrees of male fertility between this and the upper limit will need to be detected in some other way, and this may be important in discovering the cause of differential response to freezing of semen among bulls with fairly similar non-return rates with liquid semen.

## Evaluation of Semen by Chemical Analysis

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T. MANN

Whereas the early investigations on semen were chiefly concerned with morphological aspects, the more recent developments are the outcome of studies in several other fields of science, among them biochemistry. The voluminous literature pertaining to the subject of biochemistry of semen, including the secretions of male accessory organs, has been reviewed in recent years on several occasions, both in a monograph (Mann, 1954) and in review articles (Mann and Lutwak Mann, 1951, Mann, 1959). Data pertaining to more important chemical substances that occur in semen are summarized in Table III.

The purpose of this chapter is to give a brief account of chemical methods used at present in the evaluation of semen, and to discuss in general the problem of assessing the male reproductive functions by means of the biochemical procedures.

### SPERMATOZOA

The two components of whole semen, that is, spermatozoa and seminal plasma, each possess a very characteristic chemical composition. The main chemical constituents of the spermatozoa are deoxyribonucleoprotein, in the sperm nucleus, protein-bound muco

polysaccharide, in the aerosome; aldehydogenic lipid (plasmalogen), in the midpiece and tail; keratin-like proteins, which compose the sperm membrane and fibrils; and a variety of enzymes and coenzymes, which control the motility and metabolic activity of spermatozoa.

**Deoxyribonucleoprotein.** The sperm nucleoprotein is composed of deoxyribonucleic acid linked to a basic protein. Although present in the sperm nuclei of all species, the deoxyribonucleic acid is not identical in every species; it differs significantly with regard to the proportion of the four main bases, namely, adenine, guanine, cytosine, and thymine. Similarly, the protein to which deoxyribonucleic acid is bound, although generally rich in basic amino acids, particularly arginine, differs from one species to another. Moreover, recent evidence indicates that some deviations from the normal content and composition of the sperm deoxyribonucleoprotein occur in spermatozoa from subfertile individuals.

The determination of nucleic acid in spermatozoa can be carried out by several methods; the most commonly used is that based on the analysis of nucleic acid phosphorus content (Schmidt and Thannhauser, 1945; Schneider, 1945). The recent application of microspectrophotometry to semen analysis has led to a micromethod capable of determining accurately the amount of deoxyribonucleic acid in single spermatozoa (Leuchtenberger and Leuchtenberger, 1958).

**Acrosomal Polysaccharide.** The aerosome contains a characteristic protein-bound polysaccharide which is responsible for the periodic acid-Schiff staining reaction (PAS) of spermatozoa. Chemical analyses of this polysaccharide have been confined, so far, to a few species only. They show that the four main sugar components of the acrosomal carbohydrate are fucose (a methylpentose), galactose, mannose, and hexosamine. The function of the acrosomal mucopolysaccharide is probably linked with structural rather than metabolic features of the sperm cell, and may be related to the characteristic "swelling" changes which occur in the aerosome as a result of "cold-shock" or "senescence."

**Plasmalogen.** The predominant intracellular lipid of spermatozoa is of an aldehydogenic nature, namely, a choline-containing plasmalogen. This plasmalogen produces a fatty aldehyde on hydrolysis and is responsible for the so-called plasmal reaction (staining reac-

tion with Schiff's fuchsin-sulfurous acid reagent) characteristic of spermatozoa. Recent studies indicate that plasmalogen may be the substrate for the so called endogenous respiration of spermatozoa. Lecithin, which at one time was credited with being the main lipid component of the sperm cell, is now known to be more or less completely absent from spermatozoa. Some cholesterol is associated with the sperm plasmalogen, and so are small quantities of certain other steroids.

The procedure for the determination of plasmalogens in spermatozoa is based on the reaction of fatty aldehyde with the Feulgen reagent. The lipid solution prepared by extraction of spermatozoa with chloroform-methanol or propan-2-ol, is evaporated to dryness, the residue dissolved in acetic acid, and this solution treated for colorimetric determination with Feulgen's magenta reagent. The plasmalogen content is calculated from the values obtained in the colorimetric determination, with palmitaldehyde as standard (Hartree and Mann, 1959).

**Keratin like Proteins** In addition to the basic protein associated with deoxyribonucleic acid, which is confined to the sperm nucleus, the spermatozoa contain other proteins, among them a keratin-like substance which occurs in the sperm membrane and fibrils, and which is characterized by a high sulfur content. This keratin-like protein, which as yet has not been studied to any great extent, may be responsible for the remarkable elasticity of the sperm structure.

**Enzymes and Coenzymes** Spermatozoa contain a large variety of enzymes of hydrolytic and oxidative nature. Some of them, such as, for example, those of the glycolytic cycle, are bound firmly to the sperm structure. Others, for instance hyaluronidase, are readily released by spermatozoa into the surrounding medium and are assumed to be located close to the cell surface. The list of enzymes connected with the respiratory activity of spermatozoa includes succinic dehydrogenase, which oxidizes succinic acid to fumaric acid, sorbitol dehydrogenase, which oxidizes sorbitol to fructose, L-amino acid oxidase, which catalyzes the oxidative deamination of certain L-amino acids, and produces free ammonia and hydrogen peroxide as oxidation products, lactic dehydrogenase, responsible for the reversible oxidoreduction of lactic acid to pyruvic acid, and a number of other oxidizing enzymes, which utilize various fatty acids as substrates.

The cytochrome system of spermatozoa, which plays a pivotal role in the oxidative sperm metabolism, is probably concentrated mainly in the mitochondrial sheath (broad helix) of the middle piece. The components of that system consist of cytochromes *a*, *a*<sub>3</sub>, *b*, *c*, and in certain animals also cytochrome *e*, and account for the main bulk of sperm-hematin. In this respect the spermatozoa differ markedly from other animal cells, which contain several other hemoproteins. Noteworthy is the extremely low content of catalase in spermatozoa. This hemoprotein enzyme, widely distributed in other animal tissues, is practically absent from mammalian spermatozoa. Consequently, spermatozoa cannot decompose added hydrogen peroxide, and are unable to prevent the accumulation of hydrogen peroxide, which can arise in the course of oxidative reactions and which is highly toxic to the sperm cells.

Among the coenzymes present in spermatozoa, adenosinetriphosphate and diphosphopyridine nucleotide are particularly important. The level of adenosinetriphosphate in spermatozoa is related closely to the degree of sperm motility. Diphosphopyridine nucleotide plays an important role in several oxidoreductive processes, notably in the oxidation of phosphoglyceraldehyde to phosphoglyceric acid, which constitutes an essential intermediary step in sperm fructolysis.

#### SEMINAL PLASMA

The seminal plasma, which represents the combined secretions of the epididymis, vas deferens, prostate, vesiculae seminales, and Cowper's glands, is distinguished by a high content of certain organic substances, such as phosphoryleholine, glycerylphosphoryleholine, citric acid, fructose, inositol, sorbitol, ergothioneine, and spermine, most of which are not found normally, at least not in large quantities, elsewhere in animal tissues and body fluids. Among the inorganic constituents of seminal plasma, the high content of potassium, calcium, and bicarbonate, is particularly worth noting.

**Phosphoryleholine and Glycerylphosphoryleholine.** Whereas the semen of man possesses a high content of phosphoryleholine, that of the large farm animals abounds in glycerylphosphoryleholine. Ram seminal plasma, in particular, has a high concentration of glycerylphosphoryleholine, while bull, stallion, and boar seminal plasma have a lower concentration. Glycerylphosphoryleholine originates in

several accessory organs of reproduction, but the epididymal secretion is a particularly rich source of this substance. Glycerolphosphorylcholine is fairly stable in semen, and it is not readily attacked by enzymes present in either the spermatozoa or the seminal plasma. Phosphorylcholine, on the other hand, is very quickly dephosphorylated in human semen, mainly as a result of the action of the phosphatase generated by the prostate gland. The enzymic breakdown of phosphorylcholine, which occurs in human semen after ejaculation, leads to the formation of free phosphoric acid and choline. The phosphoric acid thus liberated enters into further chemical reactions, for example, it combines with spermine to form crystalline spermine phosphate. Unlike human semen, the semen of farm animals contains little or no spermine. Glycerolphosphorylcholine and phosphorylcholine can be determined in semen either as choline (after hydrolysis) or as organically bound acid-soluble phosphate, they can be further separated by chromatographic analysis (Dawson, *et al*, 1957).

**Citric Acid.** In man, citric acid is produced chiefly in the prostate gland, in the bull, boar, and stallion it is derived mainly from the seminal vesicle secretion. Citric acid is not utilized as a nutrient by the spermatozoa. Its role in the seminal plasma is not altogether clear, but it is conceivable that it is responsible, to some extent, for the high calcium binding capacity of seminal plasma.

The colorimetric determination of citric acid in semen, which requires no more than 0.1 ml. semen, is carried out in protein-free filtrates obtained by deproteinization with trichloroacetic acid. The method depends on the oxidation of citric acid to pentabromoacetone, which is extracted with petroleum ether and treated with sodium sulfide. The reaction product which results from this treatment has a strong yellow color, and can be determined either by direct colorimetry or photometrically (Speck, *et al* 1946, Mann, *et al*, 1957).

**Fructose.** The occurrence of fructose as the chief seminal sugar was first reported in bovine and human semen. The finding has now been extended to a wide range of mammals, including the ram, goat, boar, guinea pig, rabbit, rat, mouse, deer, mole, and hedgehog. In most animal species, fructose is produced in the seminal vesicles or in closely analogous glands. But in the testis and in the epididymis there is no fructose. The spermatozoa first come in contact with

fructose during the ejaculation process, that is, at the moment when the seminal vesicle secretion is voided. Once it reaches spermatozoa, fructose diffuses into the cells, and this marks the onset of fructolysis, a process which starts with fructose, continues through a number of intermediary reactions, and ends with the formation of lactic acid.

The rate of fructolysis can, therefore, be assessed in two ways, either by measuring the amount of fructose which is used up in the course of fructolysis or, alternatively, by the determination of lactic acid, which is formed as the end product. These determinations require 0.02 to 1 ml. of semen, according to the species and experimental conditions. Fructose is best determined in protein-free filtrates obtained from semen after the removal of proteins either with zinc sulfate and sodium hydroxide or with zinc sulfate and barium hydroxide or with ethanol. The actual colorimetric or photometric determination depends on color reactions of fructose with keto reagents, such as resorcinol or diphenylamine (Mann, 1948). Lactic acid can be determined colorimetrically in protein-free filtrates obtained by deproteinization of semen either with zinc sulfate and barium hydroxide or with trichloroacetic acid (Barker and Summer-son, 1941).

**Inositol.** A high concentration of inositol is characteristic of boar semen and is due to the seminal vesicle secretion. Semen of species other than the boar also contains inositol in the seminal plasma but in much smaller concentrations. A sensitive method whereby inositol can be determined in semen is a microbiological one. The technique depends on measuring the growth of the yeast *Kloeckera brevis*; the growth rate of this yeast is proportional to the amount of inositol added to the culture medium, and is assessed turbidometrically (Campling and Nixon, 1954; Hartee, 1957). The bulk of inositol present in the seminal plasma occurs in *free* form. In addition, however, both the spermatozoa and the seminal plasma contain a small amount of *bound* inositol.

**Sorbitol.** This sugar alcohol, which is chemically closely related to fructose, occurs in human seminal plasma and in the plasma of animals, such as the bull, boar, ram, and stallion. Its concentration can be determined by a combined enzymic-spectrophotometric method based on the use of an enzyme, the sorbitol dehydrogenase (King and Mann, 1958). Intact spermatozoa are capable of oxidizing sorbitol to fructose. The oxidative conversion of sorbitol to fructose

TABLE III: CHEMICAL COMPOSITION OF SEMEN  
(Expressed in mg. per 100 ml., unless otherwise stated)

	Men		Bull		Ram		Bear		Stallion	
	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range
Volume of ejaculate (ml.)	3.5	(2-8)	4.0	(2-10)	4.0	(0.7-2)	2.10	(1.50-5.00)	7.0	(3.0-30.0)
Sperm density (thousand/ml.)	100	(50-150)	1,000	(500-2,000)	3,000	(800-4,000)	100	(23-1,000)	120	(50-800)
Dry weight (gm./100 ml.)	8	(5-10.9)	9.5		14.2		4.6	(2.2-6.2)	2.4	
Chloride (Cl)	155	(100-203)	374	(309-433)	37		356	(258-428)	204	(81-443)
Sodium	251	(210-310)	109	(37-204)	103		646	(250-837)	68	
Potassium	89	(66-107)	268	(150-415)	11		245	(83-382)	62	
Calcium	33	(21-28)	34	(21-43)	9		3	(2-6)	70	
Magnesium	14		12		3		11	(5-14)	3	
Inorganic phosphorus	11		5		12		2		17	
Total nitrogen	915	(500-1,215)	756		873		813	(334-765)	167	
Non-protein nitrogen	73	(53-107)	46		44		22		15	
Urea	72		4		6		3		3	
Uric acid	6		2.5	(0.8-4)	2		3		—	(0.3-2.4)
Ammonia	2		2		2		1.5	(0.5-2)	1.3	(0.9-4.5)
Fructose	224	(91-520)	340	(780-770)	247		12	(3-48)	12	(9-15)
Lactic acid	35	(20-50)	29	(15-45)	34		27		50	(30-110)
Glucic acid	276	(100-1,430)	720	(340-1,160)	137		159	(36-325)	17	(12-28)
Total phosphorus	112		82		257		24		11	(14-22)
Acid-soluble phosphorus	37	(27.5-93.5)	35		171		6		—	
Lipid phosphorus	6		9		29		171	(110-240)	28.15	
Glycerophosphorylcholine	70	(40-100)	350	(110-490)	1,650	(1,100-2,100)	0 or		0 or	
Phosphorylcholine	—	(70-2,000)	trace		trace		trace		trace	
Erythronine	1	(0.2)	0 or		0 or		13	(6-50)	7.8	(5.5-13.7)
Inositol (free)	35	(25-46)	trace		trace		322	(382-625)	31	(15-127)
Sorbitol	10		35	(25-46)	12	(7-18)	40	(6-18)	—	(37-60)
Acetic acid	13	(11-14)	73	(10-156)	22	(28-120)	—		—	
Creatine	17		6	(3-8)	3	(2.8)	—		3	
Creatinine	—		3		2		—		9	
Carbon dioxide (ml./100 ml.)	—	(41-60)	12		—		50		24	
	—		16		16					

Source. The data given in the table are derived mainly from the author's book, *Bacteriology of Semen* (1934) but are also derived from some more recent publications.



provides the spermatozoa with an additional amount of substrate for fructolysis and lactic acid formation.

**Ergothioneine.** This sulfur-containing reducing base (betaine of thiolhistidine) was discovered in boar semen but was later shown to occur also in the semen and male accessory gland secretions of the stallion and certain insectivores. Owing to the presence of sulfydryl groups, ergothioneine is capable of reducing dichlorophenol-indophenol, iodine, and silver nitrate in the cold. It probably acts by protecting the protein-bound sulfydryl groups of spermatozoa from oxidation.

Ergothioneine can be determined by several methods, but the most convenient one depends on the color reaction with the diazo reagent (Hunter, 1951; Mann *et al.*, 1957).

#### APPRAISAL OF MALE REPRODUCTIVE FUNCTIONS BY THE CHEMICAL ANALYSIS OF SEMEN

The quantitative analysis of the various chemical constituents of semen can be conveniently used for the assessment of the gametogenic and androgenic function of the testes, as well as for the evaluation of a number of other physiological processes associated with the normal activity of the spermatozoa and male accessory organs of reproduction. The examples referred to below are based on our observations concerning the chemical characteristics of the semen of ram, bull, boar, and stallion.

**Contribution Made by Individual Accessory Organs of Reproduction Toward the Makeup of Whole Semen.** A characteristic feature of semen, as ejaculated, is the variability of its composition. This is due mainly to the fact that the contribution made by the sperm and the various accessory secretions to the entire ejaculate is by no means constant but varies greatly even in the same individual. Estimations of sperm concentration in ejaculated and epididymal semen have made it possible to assess the contribution of the epididymides toward the volume of the whole ejaculate. Similarly, it is possible to evaluate the epididymal contribution by the chemical analysis of glycerylphosphorylcholine in ejaculated and epididymal semen of bull, boar, and stallion, which, as pointed out earlier, is derived largely, though not exclusively, from the epididymal secretion.

By the use of chemical determinations of fructose and citric acid in ejaculated semen and in the vesicular secretion of bull and boar, it has been possible to calculate that the seminal vesicles contribute not less than 20 per cent, and occasionally more, toward the volume of the whole ejaculate. In the stallion ergothioneine is produced mainly in the ampullae, and citric acid mainly in the seminal vesicles, chemical determinations of these two substances have provided means for the evaluation of the contribution of the ampullar glands and seminal vesicles, respectively.

*Sequence with Which the Different Fractions of Semen Are Formed and Ejaculated.* Both in man and in animals, semen ejaculation entails the voiding of different fractions which follow one another in a definite order of sequence. Chemical analysis of semen makes it possible to determine from which part of the reproductive tract the various fractions have originated. Studies of this kind have been performed on the semen of man, bull, boar, and stallion, they indicate that the vesicular secretion is delivered usually immediately after the spermatozoa. Thus a defect in the normal sequence of the ejaculatory process or an incomplete ejaculation can be detected and assessed quantitatively by the chemical analysis of semen. Similarly, the absence or occlusion of secretory ducts which provide the normal urethral outlet for the various accessory organs of reproduction can be diagnosed chemically by analysing the contents of the seminal constituents which are normally produced in those glands.

*Chemical Methods of Semen Analysis in the Evaluation of Androgenic Activity.* Older methods for assessing the effect of androgens on the prostate, seminal vesicles, Cowper's glands, and epididymis depended on measuring the size and weight, as well as the morphological characteristics of these glands. However, examinations of this kind can only be carried out post mortem, and are, therefore, of limited value, particularly in large animals. This difficulty of evaluating the androgenic activity in the living animal can be overcome by utilizing the secretory function of the male accessory organs as reflected in the chemical composition of ejaculated semen.

The principle underlying this method is based on the numerous experiments carried out with mice, rats, rabbits, bulls, and boars in which it was established that substances such as fructose, citric acid, and ergothioneine disappear from the seminal plasma after gonadectomy or hypophysectomy, but reappear promptly in re-

sponse to testosterone administration, whether by implantation or injection. Subsequent studies, particularly with bulls, have provided ample proof for the existence of a close interdependence between the level of such substances as fructose and citric acid in semen and the activity of the male sex hormone in the living animal. However, it cannot be emphasized too strongly (cf. Mann, 1956) that, in spite of the close correlation between the concentration of certain chemical substances in semen and the androgenic activity, there are many factors, apart from the testicular hormone, which can profoundly influence the process of seminal plasma formation in the accessory organs. First of all, one must remember that the output in semen of substances such as fructose, citric acid, or ergothioneine is dependent on the actual size and storage capacity of the respective organs in which these substances are produced. Blood supply and nervous stimuli can play an important role in determining the level of secretory activity in the accessory organs. Frequency of ejaculation is another factor which must be taken into account, since frequently repeated collections of semen are bound to deplete, to some extent, the accessory organs of their secretions.

**Effects of Malnutrition on Male Reproductive Functions.** Analysis of semen can provide, up to a point, a convenient method for quantitative assessment of changes produced by underfeeding or malnutrition in the gametogenic and androgenic function of the testes. As an example, one may mention the recently conducted investigation on identical, male twin calves (Davies, *et al.*, 1957) which showed that underfeeding produces a delay of several months in the appearance of fructose and citric acid in the seminal plasma, though it causes less delay in the onset of spermatogenesis. It was possible to prove that this deleterious effect of inadequate nutrition on the secretion of fructose and citric acid in bovine seminal vesicles is due to the absence of adequate stimulation of the maturing testes by the gonadotrophic hormone and, consequently, to a lack of androgenic stimulation of the seminal vesicles. Injections of chorionic gonadotrophin to the underfed calves cause an immediate appearance of fructose and citric acid in semen.

**Chemical Criteria for the Rating of Sperm Quality.** As yet, we possess no data on a single chemical component of semen which alone could be used for the assessment of the fertilizing ability of spermatozoa. On the other hand, there exist several chemical and

physical methods which now make it practicable to rate accurately sperm motility. Most of the chemical methods available at present depend on measuring fructolysis and respiration, the two chief metabolic processes in semen, which are correlated largely with sperm density and motility.

Fructolysis, that is, the progressive disappearance of fructose accompanied by the formation of lactic acid, can be measured in whole semen, suitably diluted, buffered, and incubated at 37° C (Mann, 1948). In the absence of oxygen, semen depends on fructose as the chief source of metabolic energy, and under such conditions normal bull spermatozoa metabolize fructose at a rate of approximately 2 mg/10<sup>9</sup> motile cells per hour, at 37° C. Fructose is not utilized, or only poorly, either by azoospermic or by necrospermic semen.

It must be emphasized, however, that the rate of fructolysis, although closely correlated with the degree of sperm motility, does not necessarily reflect the fertilizing capacity of spermatozoa. This point is well illustrated by the results of an experiment carried out with the semen of a completely infertile Guernsey bull, the ejaculates of which contained only decapitated spermatozoa, that is, with heads completely separated from the midpiece-tail portions. By subjecting the semen of this bull to low speed centrifugation it was possible to obtain two fractions, one consisting of immobile sperm heads and the other of head free, yet perfectly motile, tails. However, when the rate of fructolysis was measured it was found to be high in the tail fraction and well correlated with the density of the motile tails.

Like fructolysis, sperm respiration is correlated, although less strictly, with the motility of spermatozoa. Unlike fructolysis, however, the respiratory activity of spermatozoa does not entirely depend on the presence of a substrate in the external medium, that is, the seminal plasma; it takes place as well in washed sperm after the seminal plasma has been removed from the spermatozoa. Moreover, it is possible to create, experimentally, conditions under which sperm respiration can be dissociated from motility. For instance, in the presence of fluoride, which inhibits both fructolysis and motility, the oxygen uptake of spermatozoa still continues, although it is markedly reduced.

In addition to fructolysis which can be followed by colorimetric determinations of either fructose or lactic acid, and to respiration,

which is usually determined manometrically, there are certain other processes which can be measured chemically and used in the evaluation of sperm metabolism and motility. In this category is the popular "methylene blue reduction test," which is the outcome of dehydrogenase activity of the semen and depends on the determination of the time taken by a semen sample to reduce (decolorize) a definite quantity of added methylene blue.

#### CONCLUDING REMARKS

The impact of biochemical methods upon research concerned with the physiology of spermatozoa has been gaining momentum, and one can confidently expect further developments in the application of chemical methods to the appraisal of semen quality and male fertility. When evaluating the chemical findings in semen, it is, however, important to remember that the composition of semen is subject to considerable variations; and also that an analysis of semen, even if restricted to a single experimental animal and carried out under standard conditions, need not always yield the same results. It is also wise to refrain from prematurely assigning to every chemical constituent of semen a major function in male fertility, and to every observed change in semen metabolism a deep significance in the process of fertilization. On the whole, the metabolic processes in semen are more often related to sperm density and motility than they are to the fertilizing power of spermatozoa.

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## The Role of Hormones in Reproduction

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In order that a dairy cow return the greatest profit to her owner, she should calve first at about two years of age and thereafter every twelve months during her reproductive life. To make this possible it is necessary that the reproductive systems of the cow and the bull function normally. The rapid increase in the number of cows inseminated artificially each year makes it exceedingly important that dairymen obtain additional information on the physiology of reproduction in dairy cattle.

The early investigations on the physiology of reproduction were concerned primarily with an attempt to determine the role played by the nervous system. This was logical since at that time the nervous system was the only known coordinator of bodily functions. Although the work showed that the nervous system was not primarily concerned in reproduction, nevertheless the work laid the foundation for the hormonal theory which was soon to be propounded. In the last twenty years our knowledge has been greatly increased concerning the part played by hormones in reproduction.

## ENDOCRINE GLANDS AND THEIR SECRETIONS

Glands in the body can be placed into one of two groups, depending on the manner in which the secretion is carried from the gland. The glands in one group elaborate an external secretion, and the cavities of these glands open freely on the surface from which they are derived. Such glands are called exocrine glands. The other group is made up of glands that empty their secretions directly into the blood stream and they are termed endocrine glands. The secretions of the endocrine glands, referred to as hormones,\* play a most important role in reproduction.

Of the endocrine glands, the pituitary gland, the ovaries, the testes, the adrenals, and the thyroid gland are the ones that are intimately concerned with the physiology of reproduction. The placenta, although not usually classified as an endocrine gland, produces several hormones.

**The Pituitary Gland** In cattle the pituitary gland is located ventral to the brain and in a depression in the base of the skull termed the sella turcica. The sella turcica is situated in the sphenoid bone, in the midline and about equidistant from its anterior and posterior sutures. Dorsal to the pituitary, and between it and the brain, is a tough cartilaginous membrane the diaphragma sellae. Anteriorly the pituitary stalk passes through the diaphragma sellae and connects with the brain just posterior to the union and crossing of the two optic nerves. The location of the pituitary gland in the goat is similar to that in the cow, and this is shown in Figures 14 and 15.

As one might anticipate, the weight of the pituitary gland increases as an animal matures.† This increase in weight of the pituitary is illustrated in Table IV.

The pituitary gland is composed of two major parts which are of importance in reproduction. These are the anterior lobe and the posterior lobe. In the embryo the anterior lobe and the posterior lobe develop from different regions, nevertheless, at the end of embryonic development the anterior lobe partially envelops the posterior lobe to constitute the pituitary gland. The anterior lobe

\* The term hormone means "to set in motion," "to arouse," "to excite."

† It should be pointed out, however, that gland size is not always a measure of gland activity.



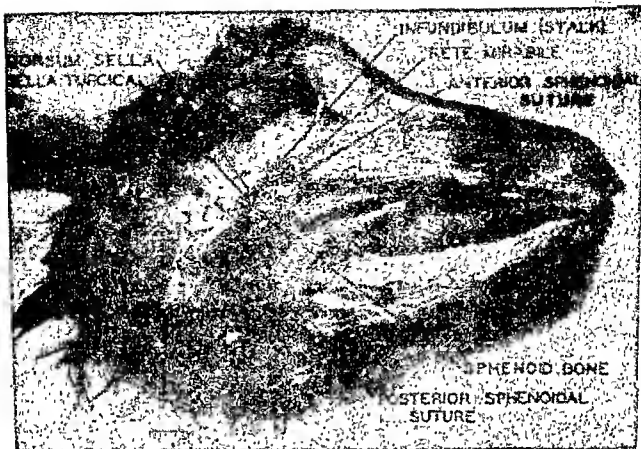


Figure 14. Sagittal section of goat head. The hypophysis has been removed, exposing the rete mirabile. (Missouri Agr. Expt. Sta. Res. Bul. 230.)

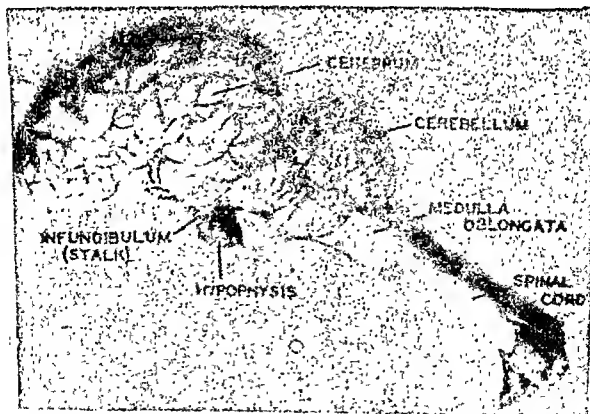


Figure 15. Lateral view of goat brain showing pituitary attached by stalk to brain. (Missouri Agr. Expt. Sta. Res. Bul. 230.)

TABLE IV TOTAL AND ANTERIOR LOBE WEIGHT OF THE BOVINE PITUITARY GLAND

Group	No of glands	Av whole pituitary weight (grams)	No of glands	Av anterior pituitary weight (grams)
Fetal	51	0.0431	22	0.0478
56-140 days	32	0.0225	7	0.0206
141-283 days	19	0.0733	5	0.0605
Calf				
Up to and including 3 months	22	0.6485	22	0.4962
4 to 11 months	234	0.9025	234	0.6973
Heifers	139	0.8898	139	0.6883
Steers	67	0.9609	67	0.7392
Bulls	28	0.8254	28	0.6425
11 to 24 months	163	1.1590	163	0.9258
Heifers	114	1.1380	114	0.9050
Steers	42	1.2104	42	0.9787
Bulls	7	1.1926	7	0.9458
Cows 2 years and over	72	1.7867	72	1.4962

Source: Reece and Turner, 1937

is composed of groups and columns of epithelial cells which are supported in a delicate connective tissue. There are two general types of cells, the chromophils and the chromophobes. The chromophil cells are usually subdivided into basophils and acidophils. The posterior lobe remains connected with the brain, from which it is derived, and it is composed almost entirely of spindle shaped and irregularly shaped cells provided with delicate processes (pituicytes).

With the unfolding of the field of endocrinology, it becomes more and more apparent that the tiny gland located at the base of the brain is the integrator of endocrine functions. Furthermore, certain nervous stimuli have their route of action via the pituitary gland. It is believed the pituitary gland secretes a number of hormones. In the physiology of reproduction our interest centers mainly on five hormones secreted by the anterior lobe and one or possibly two secreted by the posterior lobe of the pituitary gland. Those of the anterior lobe are (1) the follicle stimulating hormone (conveniently

referred to as FSH), which causes the ovarian follicle to ripen and stimulates formation of spermatozoa in the testicles; (2) the luteinizing hormone (referred to as LH), which, in conjunction with FSH, stimulates the ovarian follicles to secrete estrogen and rupture (ovulate), stimulates the secretion of androgen by the Leydig cells of the testis, and, by itself, causes the formation of the corpus luteum (yellow body) in the ovary; (3) the lactogenic hormone (also termed lactogen), which induces milk secretion in suitably prepared mammary glands and stimulates the functional activity of the corpus luteum, that is, it stimulates the corpus luteum to secrete progesterone; (4) the adrenotrophic hormone, which stimulates the cortex (outer region) of the adrenal gland; and (5) the thyrotrophic hormone, which stimulates the thyroid gland. A hormone of the posterior lobe of the pituitary gland that is concerned in the physiology of reproduction is oxytocin, which causes contraction of the smooth muscle of the uterus. Since posterior lobe extracts, both oxytocin and the vasopressor principle, can cause the contraction of the myoepithelial cells of the mammary gland, it is perhaps best to refer to the active agent simply as pituitrin.

**The Adrenal Glands.** The adrenals lie just anterior to each kidney. In the dairy cow the average weight of the adrenal glands is 31.4 gm. (Swett *et al.*, 1937).<sup>\*</sup> The adrenals are believed to have the richest blood supply of any gland in the body. They consist of two parts, the cortex and the medulla, which are distinct in origin and structure. In mammals the cortex is of mesodermal origin, but the medulla is derived from the ectoderm. Structurally, the cortex consists of cells with many sides arranged in three zones. The medulla consists of irregularly shaped cells in relation to large vascular spaces, and the cells stain dark brown with chromic acid; therefore, it is sometimes referred to as chromophilic or chromaffin tissue. It is the cortex of the adrenals that is of interest to us in the physiology of reproduction, especially lactation. As a matter of fact, the medulla can be spared without endangering life or interfering with normal existence.

A number of hormones have been obtained in pure form from the adrenal glands, and several of them have been synthesized in the laboratory. Since none of the isolated hormones furnishes complete replacement therapy for the adrenal glands, whereas an ex-

<sup>\*</sup> This figure is an average computed from the breed averages reported by Swett *et al.*

tract of the glands does, we shall refer to such an extract as the adrenal cortical hormone. The adrenal cortical hormone influences the body in a number of ways. In lactation, however, we are primarily concerned with the role it plays in the handling of carbohydrates in the body and its regulation of the sodium and water content of the blood. Although it is not known what part the hormones of the adrenal gland may exert in the control of the estrous cycle and in pregnancy, it is possible that they may have a supplementary action. Androgenic hormones have been isolated from the adrenal cortex, and, at times, early sexual maturity and masculinization in the mature female are associated with tumors of the adrenal cortex.

**The Thyroid Gland** The thyroid gland consists of two lateral lobes which are situated on each side of the trachea either near to or in contact with the larynx. The two lateral lobes are usually joined by an isthmus. The gland has an abundant blood supply, is firm in texture, and is pale red in color. In the dairy cow the average weight of the thyroid gland is 32.9 gm (Swett *et al*, 1937).<sup>\*</sup> The thyroid gland develops from the floor of the pharynx and therefore originates from the endoderm. Thyroid tissue consists of spherical vesicles which are lined by a single layer of squamous or columnar epithelial cells, depending upon the activity of the gland. The lumina or openings of the vesicles are filled with a viscid substance termed colloid which contains the thyroid hormone in combination with a protein. The hormone of the thyroid gland is present in thyroglobulin which contains thyroxine, and its action contrasts with that of the other hormones in that its effect is general in nature rather than specific. Thyroxine stimulates cellular activity, and associated with this increase in cellular activity is an increase in basal metabolic rate. In addition, thyroxine administration increases heart rate, and this in turn results in a greater blood supply to the cells. Thyroxine can be synthesized in the laboratory and a substitute for it is now available in the form of thyroprotein.

**The Ovaries** † In the dairy cow the average weight of the ovaries is 22.7 gm. ‡ The ovaries have a dual purpose: the production of eggs

<sup>\*</sup> This figure is an average computed from the breed averages reported by Swett *et al*.

† Refer to Chapter 2 for description.

‡ This figure is an average computed from the breed averages reported by Swett *et al*.

or ova and the secretion of three hormones: (1) an estrogen, (2) progesterone, and (3) relaxin. Estrogens induce growth of the vagina, uterus, Fallopian tubes, and the duct system of the mammary glands. They also begin the preparation of the lining of the uterus for the reception of the fertilized egg. Progesterone completes the preparation of the uterus for the fertilized egg, keeps the uterus in a quiescent state during pregnancy, and in conjunction with estrogen it induces the development of the lobule-alveolar system of the mammary glands. Relaxation of the pubic ligaments at the time of parturition may be dependent upon the action of relaxin.

**The Testes.** The testes, like the ovaries, perform two major functions. They produce spermatozoa and secrete androgens. The androgen secreted by the testis is probably testosterone and has been identified as such in the bull, stallion, and boar. Androgens elicit growth of the genitalia and maintain them in a functional state. They produce secondary sexual characteristics, such as the crest in a bull's neck, and stimulate sex drive. Androgen may also aid the testes in the formation of spermatozoa.

**The Placenta.** It was indicated earlier that the placenta is not usually classified as an endocrine gland, but it does secrete several hormones. The placenta of primates secretes a gonadotrophic hormone whose action is similar to, but not identical with, that of the luteinizing hormone of the anterior lobe of the pituitary gland. This hormone secreted by the primate placenta is usually referred to as chorionic gonadotrophin. In the mare the endometrial cups of the uterus secrete a gonadotrophic hormone which can be obtained from the blood serum and is commonly called pregnant mare serum or PMS. The action of PMS on the gonads is intimately related to dosage. The placenta secretes considerable quantities of estrogen, and there is some evidence that it secretes progesterone. It secretes another hormone, the luteotrophic hormone, which maintains the functional activity of the corpus luteum. It would appear, therefore, that the main hormonal function of the placenta is to induce the production of progesterone by the corpus luteum and to secrete progesterone, which supplements or replaces that secreted by the corpus luteum.

## HORMONES REGULATING REPRODUCTION

It was known many years ago that certain glands in the body were able to influence other parts of the body by secreting chemical substances and discharging them into the blood. Berthold (1849) showed that the testis was not dependent upon specific nerves to maintain its control of the secondary sex characters, but that its influence was exerted through the blood. Knauer (1900) showed that grafted ovaries prevented the occurrence of castrate atrophy.

Experimental evidence began to accumulate in 1897 (Turner, 1933) demonstrating that the nerves were not primarily concerned in stimulating the growth of the mammary gland and in the initiation of lactation. A pituitary-gonad relationship was suggested by Fichera's work (1905), which showed that the pituitary gland enlarged after spaying or castration in guinea pigs, rabbits, cattle, buffaloes and cocks. It remained, however, for P. E. Smith (1926, 1927) to demonstrate conclusively that the anterior lobe of the pituitary gland maintained and controlled the activity of the ovaries and testes and as a result indirectly influenced other reproductive organs. Our knowledge of the role played by ovarian and testicular hormones is well founded.

## HORMONES IN THE FEMALE

*The Estrous Cycle* As an animal approaches sexual maturity the anterior lobe of the pituitary gland secretes increasing amounts of the follicle stimulating hormone and thus in turn brings about the maturation of one or more ovarian follicles, depending upon the species. The mature follicle designated a Graafian follicle, contains liquor folliculi in which estrogen can be detected. In the sow both estradiol  $17\beta$  and estrone have been isolated from the ovaries. A small amount of luteinizing hormone in conjunction with FSH induces the secretion of estrogen by Graafian follicles. The action of estrogen makes the female receptive to the male and we say that she is in estrus. In addition the estrogenic hormone stimulates vaginal growth and also causes the cervical secretions to become more fluid so that during estrus a clear fluid discharge from the vagina is often observed. Indeed the characteristics of cervical secretions

have been utilized for the detection of estrus in cattle (Scott Blair *et al.*, 1941). Estrogen induces uterine growth and begins the preparation of the uterine lining (endometrium) so that it will be in a suitable condition to receive the fertilized egg (zygote).

Estrogen reacts on the anterior lobe of the pituitary gland and suppresses the discharge of FSH. It brings about, however, the discharge of the luteinizing hormone, which, in conjunction with FSH, causes the ovum or ova to be shed from the ovary; that is, it induces ovulation. The results of recent work in dairy heifers suggest that progesterone may play some role in ovulation (Hansel and Trimberger, 1951). The injection of 5 to 10 mg. of progesterone at the beginning of estrus resulted in a reduction of time from the end of estrus to ovulation. The responses to progesterone treatment were somewhat variable, however, the average time elapsing between the end of estrus and ovulation was only half as great in the progesterone-treated periods as in the control periods. Ovulation is the most important event in the estrous cycle, and it usually occurs near the end or shortly after the termination of estrus, depending upon the species.

When an animal is to be inseminated, the insemination should be done prior to the time of ovulation. It was once believed that it required four or five hours for spermatozoa to reach the Fallopian tube and that this travel time accounted for the need to inseminate prior to ovulation. Evidence is now available, however, indicating that spermatozoa arrive in the ampulla of the Fallopian tube within five minutes after insemination. This necessity for insemination before ovulation is now explained on the basis that spermatozoa acquire the ability to fertilize ova after deposition in the female tract. This phenomenon is referred to as capacitation and requires about six hours in rabbits and rats. Following ovulation, ova retain their ability to be fertilized for only a short period, and the life of sperm following insemination is somewhat longer.

A new type of tissue, lutein tissue, forms in the Graafian follicle after ovulation, under the stimulus of LH. This structure is termed a corpus luteum, commonly called a yellow body, which secretes progesterone. Progesterone acts on the anterior lobe of the pituitary gland and inhibits the discharge of LH in quantities sufficient to induce follicular maturation and ovulation. Therefore, as long as progesterone is present in the blood in sufficient quantities, mature

follicles do not form, and estrogen production is impeded. In addition, progesterone completes the preparation of the endometrium (progestational proliferation) for the reception of the fertilized ova.

If the animal is not mated or if conception does not occur, the corpora lutea regress, with the result that progesterone is no longer produced. There are exceptions to this statement, in the bitch ovulation takes place spontaneously, and in many instances the corpora lutea will remain functional for a period of time (a condition called pseudopregnancy) about equal to the duration of pregnancy even though the bitch is not mated. With the decrease in the level of progesterone in the blood the pituitary is freed from the inhibitory action of progesterone, and this results in the repetition of the series of events just described.

The term "breeding season" is usually used to designate the time or times of year during which the reproductive organs exhibit a special activity. Those animals that experience estrous cycles throughout the year, cattle for example, are referred to as polyestrous animals. In certain animals (sheep), estrous periods recur but for only a part of the year, and such animals are called seasonally polyestrous animals. In other animals estrus does not recur during the breeding season, and such animals are designated as monoestrous. Although estrus does not recur during the breeding season of monoestrous animals, they may have more than one breeding season each year. The dog, cat, and fox are monoestrous animals. The nonbreeding season, a time when the reproductive organs are relatively quiescent, is usually spoken of as the anestrous period. Environmental factors (light, temperature, and food) influence reproductive function in both seasonal and nonseasonal breeders. These factors stimulate the secretion and/or release of gonadotrophin from the anterior lobe of the pituitary gland, the gonadotrophin in turn activating the ovaries and testes.

Ova arise from the germinal epithelium of the ovary, and evidence has been presented (Allen and Creadick 1937) which indicates that in the mouse this may extend into the postpubertal period. In the mature ewe and cow no evidence has been found which would indicate that new ova arise from the germinal epithelium (Cole, 1933). Healthy and regressing follicles were found in the ovaries at all times in about the same proportions.

In the bovine the ovaries are not functionally equal. The right



ovary is functionally more active than the left ovary, and this difference in functional activity accounts for the fact that a greater number of pregnancies occur in the right uterine horn than in the left horn (Reece and Turner, 1938).

**Pregnancy and Parturition.** If an animal is mated shortly before ovulation and the ova are fertilized, the corpora lutea persist and secrete progesterone. In those animals in which pseudopregnancy can be induced by a sterile mating, it is probable that the nervous stimulation from mating activates the anterior lobe of the pituitary gland to secrete and discharge the lactogenic hormone (also called luteotrophic hormone), which stimulates progesterone formation by the corpora lutea. When the ova are fertilized, it is not known what causes the corpora lutea to persist in those animals in which pseudopregnancy cannot be induced by a sterile mating. In other words, how the corpora lutea "know" that the ova were fertilized remains an unsolved problem in endocrinology. It seems probable, however, that the pituitary gland is responsible for maintaining the functional activity of the corpora lutea during the first half of pregnancy, since its removal results in fetal resorption and the placenta secretes a luteotrophic factor which maintains the corpus luteum during the latter part of a pregnancy.

Progesterone completes the preparation of the uterus for the implantation of ova, and in all species studied the ovaries must remain intact if implantation is to take place. Progesterone stimulates the growth and secretion of the uterine glands, the secretion of which (uterine milk) serves as a nutrient medium for the dividing ovum until the maternal placenta and fetal placenta make contact. In the cow, it has been shown that 33 days after ovulation there is a very fragile attachment between the maternal and fetal tissue at three or four of the caruncles immediately surrounding the embryo (Melton *et al.*, 1951). In addition, placental formation and maintenance depend upon the action of progesterone and estrogen, and the two hormones must be present in proper proportions. The muscular coats of the uterus are maintained in a quiescent state by progesterone, thus preventing the uterus from discharging its products of conception.

In certain animals (guinea pig, mare, and woman) the ovaries are not necessary for the continuation of pregnancy during its latter half. In such cases it would appear that the placenta is capable of

secreting sufficient progesterone for the maintenance of pregnancy. In other animals (cow and sheep, for example) abortion results if either the ovaries or the corpora lutea are removed. When the ovaries are necessary for the maintenance of pregnancy, their corpora lutea remain in a functional state until the approach of parturition. At this time the corpora lutea regress, and the level of progesterone in the blood decreases. The placenta may, even in those animals in which the ovaries are necessary for the maintenance of pregnancy, secrete progesterone to supplement that secreted by the ovaries. For example, material exhibiting the activities of progesterone has been obtained from cow's placenta (Adler *et al.*, 1934). There is some evidence indicating that 75 mg of progesterone injected subcutaneously daily will maintain pregnancy in Holstein Friesian heifers following the removal of the corpus luteum in the early stages of pregnancy (Raeside and Turner, 1951). In goats it has been shown that 25 mg of progesterone daily adequately substitute for the corpora lutea during the last third of pregnancy (Meites *et al.*, 1948).

Animals, as a general rule, do not come into estrus during pregnancy since progesterone inhibits the discharge of LH from the pituitary gland and as a consequence mature follicles do not form in the ovaries.

In the first part of pregnancy rather small quantities of estrogen are secreted, and as pregnancy advances ever increasing amounts of estrogen are formed. In the pregnant mare large quantities of estrogen are excreted in the urine, and in the cow estrogens are excreted in the urine and feces. There is some evidence indicating that progesterone retards the rate of estrogen inactivation, and as a result increased amounts are available for body utilization and for excretion in the urine.

Estrogen in conjunction with progesterone increases the size of the uterus so that it is able to accommodate the products of conception. Uterine distention by the fetus stimulates uterine growth, and this mechanical factor appears to cooperate with the hormones, estrogen and progesterone, in bringing about the tremendous increase in the size of the uterus which occurs during pregnancy.

It has been noted that progesterone plays a part in the maintenance of pregnancy. Parturition approaches at a time when the blood level of progesterone is decreasing and this may be a factor in the initiation of parturition since progesterone is able to prevent

the action of oxytocin, a hormone of the posterior lobe of the pituitary gland, on the uterine muscle. On the other hand, the amount of estrogen excreted in the urine increases until parturition occurs. Estrogen causes rhythmic contraction of the uterine musculature and also sensitizes the uterine muscle to oxytocin, which has the ability to cause the uterine muscle cells to contract. The onset of parturition, therefore, occurs at a time when estrogen production is high and progesterone production is declining. The ratio of estrogen to progesterone is probably the determining factor, since the injection of estrogen can interrupt an established pregnancy even though progesterone is being produced. These injections change the estrogen-progesterone ratio, and as a result an abortion occurs.

A third ovarian hormone, relaxin, comes into play near the end of pregnancy. The pelvic ligaments, following their sensitization by estrogen, are caused to relax by relaxin, and this results in a widening of the birth canal, which makes easier the expulsion of the fetus. The relaxation of the ligaments around the tail head, long known to be a sign of approaching parturition, is a result of relaxin action.

**Udder Growth.** Although little glandular tissue is present in the fetal udder, its form and teat placement have been determined, and macroscopically it has the appearance of a miniature udder. At birth the primary milk ducts extend only a short distance from the gland cistern. During the prepubertal period there is a gradual extension of the duct system and an increase in adipose and connective tissue. Well-fed heifer calves may show a considerable increase in udder size; this, however, is largely a result of fat deposition.

With the onset of puberty the estrogen secreted by the Graafian follicle stimulates the growth of the duct system of the udder.\* This estrogenic action is brought about in two ways: first by direct action on the udder, and second by an indirect action. In its direct action estrogen causes more blood to flow to the udder, which may be associated with an increased permeability of the blood capillaries. This direct action in itself is not sufficient to induce duct growth, since estrogens are ineffective in animals from which the pituitary gland has been removed. Indirectly, estrogen stimulates the anterior lobe of the pituitary gland to secrete lactogen, and this hormone, cooperating with estrogen, induces duct growth. With each recurring

\* The duct system of the udder may be looked upon as the conveying system of the udder, the ducts also serve as a storage system.

estrous cycle there is additional growth of the duct system, and the extensiveness of duct development at the time of conception, within any one animal, depends upon the number of estrous cycles experienced by that animal.

In animals that have had a number of estrous periods before conception there is probably not much additional growth of the duct system, but if conception occurs soon after sexual maturity is attained there may be considerable duct growth in the early stages of pregnancy. The striking change in the mammary glands during three-quarters of pregnancy is the formation of the secretory units, the alveoli, at the ends of the terminal ducts. A group of alveoli is surrounded by a thin band of connective tissue, and such a structure is termed a lobule. In turn a number of lobules are surrounded by a thicker band of connective tissue, and such a structure is called a lobe. The lobules are usually referred to as the lobule alveolar system of the mammary gland.

The development of the lobule alveolar system of the mammary gland is brought about by two ovarian hormones, estrogen and progesterone, and a hormone of the anterior lobe of the pituitary gland. As in duct development, the action of estrogen, as well as progesterone, in lobule alveolar development is not entirely a direct action, since they fail to induce lobule alveolar growth in an animal whose pituitary has been removed. These ovarian hormones appear to stimulate the pituitary gland to secrete a hormone which acts in conjunction with them in bringing about the development of the lobule alveolar system of the mammary gland. This pituitary factor has not been identified, but it is protein in nature, and there are indications that it is not identical with lactogen, thyrotrophin, or gonadotrophin (Mixer and Turner, 1943).

Near the end of pregnancy, cellular division of the glandular tissue of the mammary glands is greatly reduced, and the cells lining the alveoli begin to secrete. This secretory activity causes the mammary glands to increase in size, and this has been responsible largely for the general belief that most of the mammary gland growth occurs just prior to parturition. This, as has been pointed out, is not the case. Estrogen, which is secreted in increasing amounts as pregnancy advances, gradually stimulates the pituitary gland to secrete and discharge small quantities of the lactogenic hormone. Lactogen in turn stimulates the secretory activity of the cells lining the alveoli.

**Lactation.** Immediately following parturition there is a great surge of secretory activity in the mammary glands. This secretory activity is induced by the discharge of lactogen from the pituitary gland, and this initial discharge is not dependent upon the stimulus of suckling. After lactation is established, the discharge of lactogen from the pituitary gland is dependent upon the stimulus of suckling or milking (Reece and Turner, 1937).

Lactogen alone will neither initiate nor maintain lactation in an animal from which the pituitary gland has been removed. There are, therefore, other pituitary hormones which play a role in lactation. The adrenotrophic hormone stimulates the cortex of the adrenal glands to secrete the adrenal cortical hormone which is necessary for lactation. Thyroxine, although it is not necessary for lactation, augments the level of milk production, and its secretion is under the control of the thyrotrophic hormone of the pituitary gland. When milking or suckling is stopped, nervous impulses no longer reach the pituitary gland to bring about the discharge of the lactogenic hormone; and as a result the secretory activity of the mammary glands soon subsides.

Milk ejection or removal is under hormonal and nervous control. Washing the udder, fore-stripping, or the milking act itself initiates nervous impulses that are conveyed to the posterior lobe of the pituitary gland, where they stimulate the discharge of pituitrin. Pituitrin is carried in the blood to the udder, where it brings about the contraction of the myoepithelium of the mammary gland. When these events occur, one says that the milk has been "let-down." It is then possible to remove all except two or three pounds of residual milk from the mammary glands.

#### HORMONES IN THE MALE

At birth spermatozoa are not present in the testis. As an animal grows, increasing amounts of pituitary FSH are secreted, and this stimulates the formation of spermatozoa. The pituitary then secretes LH, a hormone that brings about the secretion of androgen by the Leydig cells, which lie between the seminiferous tubules. If androgen secretion reaches a certain level, it reacts on the anterior lobe of the pituitary gland and decreases the formation of the gonadotrophic hormones; otherwise, the testis and the pituitary gland appear to

be in a state of equilibrium. A decrease in the secretion of gonadotrophic hormones results in the production of less androgen by the Leydig cells and fewer spermatozoa by the seminiferous tubules. The lowered level of androgen secretion permits the pituitary gland to secrete additional quantities of gonadotrophic hormones, which again stimulate androgen secretion and spermatozoa formation. Thus it is seen that testis function is controlled by the reciprocal action of the pituitary and the testis. In males that mate throughout the year, there is a rather constant rate of gonadotrophic hormone secretion. On the other hand, in seasonal breeders the rate of secretion of the gonadotrophic hormones (FSH and LH) decreases rapidly after the breeding season and remains low until the approach of the next breeding season. At this time a factor, usually environmental in nature, stimulates the pituitary to secrete FSH and LH, and these hormones enable the testis to become functional again.

In certain species the thyroid gland plays an important role in the physiology of reproduction. It has been shown that the high temperature of summer induces a decrease in the number of spermatozoa and a marked increase in the number of abnormal spermatozoa in ram semen. That the thyroid gland is involved in these changes of semen characteristics is indicated by the fact that the administration of thyroxine will increase the number of spermatozoa and decrease the percentage of abnormal spermatozoa (Berliner and Warbritton, 1937). Additional work along this line indicates that the hormone of the thyroid gland influences the spermatogenic tissue of the testis but has little or no effect on the interstitial tissue, the tissue that secretes androgen (Bogart and Mayer, 1946).

Androgen induces the growth of the accessory reproductive organs and secretory activity of the epithelium of the organs whose secretion serves as a carrier of the spermatozoa. This enables the male, at sexual maturity, to deposit the spermatozoa in the female reproductive tract or artificial vagina. Of special interest is the dependence of the scrotum on androgen for its complete development and maintenance. The scrotum serves as a thermo regulatory mechanism for the testis, maintaining the testis at a temperature several degrees below body temperature. In those animals that maintain the testes outside of the body, sperm formation ceases if the testes are anchored in the abdominal cavity. Such animals, however, retain their ability to mate since the testis is still able to secrete androgen. A similar

circumstance is that of a cryptorchid animal. The development of male-like characteristics is dependent upon androgen secretion, and it is possible that androgen may aid the testis in sperm formation.

Sex drive is largely controlled by androgen, but other factors enter into the picture. Some of these factors have been brought to the foreground by artificial insemination programs (Hart *et al.*, 1946). The psychical effect on bulls of nonestrous cows is much less than that of estrous cows. There is an odor from the estrous cow that attracts and psychically stimulates the bull under the conditions of artificial insemination. The resulting stimulation is so marked that many bulls that appear to be impotent breed immediately.

#### HORMONAL THERAPY IN CATTLE

Although hormones are intimately concerned in reproductive processes, hormonal imbalance is not the major factor in the reproductive failure of farm animals. Unquestionably, infection is responsible for a greater number of these failures. There are times, however, when it may be definitely advantageous to resort to hormonal therapy, and each day a greater number of persons are becoming aware of this fact. Despite a decidedly favorable physiological response to hormonal therapy, in some instances it is evident that economic considerations enter into the picture. For example, it may be economically unsound to use a certain hormone on commercial herds, whereas it may be decidedly advantageous to use the hormone on valuable breeding animals.

*Cows Failing to Conceive after Repeated Service.* From a reproductive point of view, probably the greatest loss to the dairy farmer is brought about by those cows that successfully terminate a normal gestation period only after repeated service. In repeat breeders, fertilization may or may not take place. Fertilization may fail to occur for several reasons. Ovulation and insemination may not have been properly synchronized; ovulation may occur while the cow is still in estrus, and again it may not occur until 26 hours after the cessation of estrus (Brewster *et al.*, 1940). A majority of cows, however, will ovulate from 12 to 15 hours after the cessation of estrus. Unfortunately, many dairymen are unable to utilize this information in the insemination of their cows, since they do not have the time to determine when estrus begins or when it ends. In the winter they

are probably fortunate if they are able to detect those cows that are in estrus. It is also possible that cycles may occur in the cow without ovulation (anovulatory cycles), and this is probably the result of the failure of the pituitary gland to secrete sufficient LH. Finally, luteinization of the follicle may take place too rapidly, and as a result the ovum is imprisoned in the follicle. An excess of LH appears to be responsible for such a condition.

There is some evidence that the hormonal treatment of cows showing regular estrous cycles but failing to conceive may be advantageous. A group of such cows was subjected to chorionic gonadotrophin therapy by Bottomley *et al.* (1940). The treatment consisted of three intramuscular injections of 1,000 rat units each, with the first injection being made on the day of service and the remaining injections being given at two day intervals. The services of experimental cows were classified as treated and untreated. The group of treated services included not only the services when chorionic gonadotrophin was administered but also the subsequent service, provided that the latter occurred within 28 days. Of 30 treated services, 56.7 per cent were effective, whereas only 6.9 per cent of untreated services were effective. The percentage of effective treated services of the experimental cows was similar to that of a group of normal cows.

The results of Lubin's work (1946) suggest that pregnant mare serum (PMS) is effective in cows that fail to conceive after repeated service. Twenty three cows that had failed to conceive after an average of 5.4 services were not bred on their next estrus, since it was possible that a hormonal imbalance existed. These animals were injected subcutaneously with 750 to 1,500 rat units of PMS 16 days after their last estrus, and the cows were bred on their following estrous periods. Of these 23 cows, 19 conceived: 16 after one service and 3 after two services. There were four sets of twins, one of which was aborted at five months. One cow failed to conceive after two services, one developed ovarian cysts, in one the corpus luteum persisted, and one developed pyometritis.

Other attempts to restore fertility in dairy cattle, by treatment with an estrogen and gonadotrophin, have not been successful. Cows which showed fairly regular estrous cycles but failed to conceive were brought together under a uniform system of management (Asdell *et al.*, 1942) and divided into treated and nontreated groups. The animals in the treated group were given a subcutaneous injection



tion of 20,000 or 30,000 rat units of estradiol benzoate, and, following mating, if conception did not occur, an anterior pituitary extract was injected alone. In the treated group, 42.9 per cent of the cows conceived and calved, as compared with 50 per cent in the nontreated group.

Although administered gonadotrophins appear to be effective in cows failing to conceive following repeated service, nevertheless, definite recommendations cannot be made until properly controlled experiments have been carried out.

Preliminary observations indicate that progesterone administration following insemination may increase the conception rate in cattle free from detectable infection and other reproductive abnormalities. Eighty-two treated animals had a first-service conception of 70.7 per cent, whereas 84 controls had only 46.4 per cent (Johnson, 1958).

Some studies have been conducted to determine the cause of reproductive failures in cows of low fertility (Tanabe and Casida, 1948). The reproductive performance during the first 34 days after breeding of a group of 104 cows, each of which had been bred four to thirteen times, showed: (1) fertilization failed to occur in 39.7 per cent; (2) embryonic abnormalities and mortality in 39.2 per cent; and (3) normal embryos in 21.1 per cent.

**Anestrous Cattle.** Animals that fail to come into estrus should be divided into two groups: (1) those with nonfunctional ovaries and (2) those with corpora lutea in their ovaries.

Estrogens have been used more extensively in cows with nonfunctional ovaries than perhaps any other hormone. Claims were made not only for the induction of estrus but also for the initiation of the estrous cycle. A summary (Reece, 1945) of the more recent literature indicated that estrogens were effective in anestrous cattle. Following the synthesis of a cheap synthetic estrogen by English workers, it was possible to use estrogen on a larger number of animals. The early reports on the use of this synthetic estrogen, stilbestrol, were very favorable; but it now seems certain that stilbestrol, or any estrogen as far as that is concerned, will not fulfill its early promise. Estrogens do have the ability to induce estrus in anestrous animals, but this in itself serves no purpose. The ovaries must be activated so that they produce mature follicles which will ovulate.

The missing factor in anestrous cattle appears to be FSH of the pituitary gland. The FSH output of the pituitary gland is influenced

by the nutritional condition of the animal, and it is interesting to note that anestrus is frequently observed in heifers not well fed during the winter months. Since FSH is the missing factor in such cases, a logical choice of a gonadotrophin would be one rich in FSH.

Gonadotrophic hormones have been tried in anestrus cattle. The subcutaneous injection of 1,500 international units of PMS will induce estrus and ovulation, with estrus appearing on the second or third day after injection (Bhattacharya *et al*, 1941). In fact, 1,000 international units of PMS induced estrus in 21 of the 46 cows previously exhibiting dormant estrous cycles. Larger doses should not be used, since they may cause ovarian cysts to develop.

A gonadotrophic extract prepared from the anterior lobes of horse pituitaries consists mainly of FSH, and its use in anestrus animals seems to produce satisfactory results. Its main drawback is that the supply of horse pituitaries is limited, and as a result such an extract may not be available in sufficient quantity to permit its use on a large scale.

Chorionic gonadotrophin has been used and with seemingly good results. In the early work a small dosage was used, and it may be questionable just what part the hormone played in inducing estrus. Moreover, since the action of chorionic gonadotrophin is similar to that of LH of the pituitary gland, there appears to be little justification for its use.

Following calving, a cow may fail to come into estrus, and an ovarian examination may show that one of the ovaries contains a corpus luteum. The failure of such an animal to come into estrus is usually attributed to a persistent corpus luteum. Actually, only one point has been proved, the presence of a corpus luteum. Repeated examinations would be required in order to prove that the corpus luteum was persistent. The fact that some cows experience silent estrus justifies such a statement.

Any attempts to use hormonal therapy in animals with a persistent corpus luteum should take into consideration the desirability of simulating natural conditions. In the estrous cycle the corpus luteum forms, remains functional for a while, and then regresses at a time when a new follicle is maturing. It would seem, therefore, that one should attempt to induce follicular maturation similar to that which occurs during the estrous cycle. When it is known what factor, or factors, causes the corpus luteum to remain functional during the

early stages of pregnancy, we shall be in a better position to contend with this problem.

The warnings of Bhattacharya *et al.* (1941) in using PMS in cows with corpora lutea in their ovaries are worth attention, since (1) if PMS is administered when a corpus luteum is present, ovarian cysts may form, and (2) PMS should never be injected in conjunction with the removal of the corpus luteum, as multiple ovulations may occur with the danger of multiple births. It should be emphasized that even though chorionic gonadotrophin induces estrus when an active corpus luteum is already present, the newly formed corpora lutea do not take control of the cycle (Zawadowsky *et al.*, 1935).

For the induction of estrus in cows whose ovaries contain corpora lutea it appears best to have the corpora lutea expressed manually by a competent veterinarian.

**Cystic Ovaries.** The ovary of a cow may produce a mature follicle, but it may not rupture. The follicle may then increase in size and persist, thus forming a cyst. The follicular fluid of these cysts contains estrogen, although the concentration is less than that in the fluid of normal follicles (Reece and Crowshaw, 1949). In such animals the estrous cycles become shorter and irregular; and if the condition is not corrected, the animal is likely to take on the characteristics and behavior of a bull.

It is known that LH plays a major role in the induction of ovulation, and it is believed that the ovaries of certain cows become cystic because their pituitary glands are not secreting sufficient LH. Therefore, it should be possible to correct a cystic condition by the injection of LH, and this seems to be the case. The subcutaneous injection of 10,000 international units of chorionic gonadotrophin or the intravenous injection of an extract prepared from sheep pituitaries is effective.

**Retained Placenta.** In certain herds the retention of the fetal membranes creates a serious problem. The retention of fetal membranes usually results not only in greater difficulty in getting the cows with calf but also in lowered milk production.

Since estrogens and pituitrin have the ability to induce uterine contraction, it seemed logical to use them in an attempt to expel the fetal membranes. Soon after stilbestrol became available to the veterinary profession, case reports appeared in the literature indicating the effectiveness of estrogen in bringing about the expulsion of the

fetal membranes More recent work however, indicates that estrogens are unable to expel fetal membranes which are firmly attached Moore (1946a) injected stilbestrol into 43 cows that had retained placenta A careful manual examination of the uterus was made in each case The animals were injected with 30 to 80 mg of stilbestrol when it was thought that the placenta was too firmly attached to come away by itself in 48 hours In some animals the dose was repeated from one to three times The fetal membranes were not removed manually so long as it was thought that they could be left without endangering the life of the animal In eight cases the placenta eventually came away unaided, but in the remaining 35 cases it was necessary to remove it manually

Since placental retention in the cow is usually associated with pathological changes in the cotyledons, it is not surprising that estrogens have been of little value in inducing placental expulsion

*Pyometra* Estrogens appear to be effective in cases of pyometra They dilate the cervix and stimulate uterine contraction, and these factors operate to bring about the discharge of pus from the uterus In addition, the increased blood flow to the uterus undoubtedly aids in returning the uterus to a normal condition

Forty three cows that developed pyometra following the manual removal of placenta were injected with stilbestrol by Moore (1946b) None of the placentae was removed in less than 72 hours after calving, and some remained as long as 148 hours The only other treatment consisted in the placement of a one-ounce gelatine capsule containing sulfanilamide deep in each horn of the uterus Two weeks later, at which time all symptoms of acute inflammation had subsided but in all instances pyometra was present, a single dose of from 30 to 50 mg of stilbestrol was injected Daily observations were made thereafter, and if no results were obtained in 72 hours, the treatment was repeated All cows were bred at the first estrus which occurred 60 days following parturition, and were rebred at each succeeding estrus For comparative purposes, the records of 50 cows which were in the same herds, which retained their placentae, and which were not treated with stilbestrol, were selected at random In the stilbestrol treated animals 97.8 per cent retained their fertility, as compared with 86 per cent in nontreated cows In the injected cows an average of 86.2 days elapsed from parturition to the con

ception following treatment. In the noninjected group an average of 144.8 days elapsed from parturition to the following conception.

In pyometra, where prolonged stimulation of the uterus is desirable, a dipropionate, either stilbestrol dipropionate or estradiol dipropionate, should be the estrogen of choice.

**Mummified Fetus.** If the placenta becomes nonfunctional at certain stages of pregnancy, the fetus is retained in the uterus and becomes mummified. It is believed that the fetus is retained because the blood estrogenic level is not sufficiently high to set in motion those factors operating at parturition. The injection of an estrogen, 25 mg. of either estradiol dipropionate or stilbestrol dipropionate, will usually bring about the expulsion of the mummified fetus.

**Initiation and Maintenance of Lactation.** The work on the hormonal control of lactation has clearly shown the role played by the lactogenic hormone of the pituitary gland. Copious lactation followed the injection of lactogen in animals with suitably prepared mammary glands. The withdrawal of lactogen treatment, however, resulted in cessation of lactation even in those cases where the milk was removed from the mammary glands.

In 1937 it was shown that the injection of an estrogenic hormone into ovariectomized rats not only increased the lactogenic hormone content of the pituitary gland but also initiated lactation. Later work showed that the lactation initiated by estrogenic treatment of rats could be maintained, without additional treatment, by the suckling influence of a litter. In about 50 per cent of the trials sufficient milk was secreted so that the injected foster mothers were able to rear normal litters.

The synthesis of stilbestrol by English investigators has made it possible to attempt initiation of lactation experiments with larger animals. The injection of stilbestrol into heifers usually induces growth of the udder and teats, and milk secretion. At times lactation is not initiated until the heifer is stimulated either by milking or by a nursing calf.

In the experiments with dairy heifers, the quantity of hormone used, the duration of treatment, and the mode of administration have varied greatly. The quantity of milk secreted has varied from negligible amounts to quantities that would be considered normal following parturition. A thirty-three-month-old barren Jersey heifer that received a total of 273 mg. of stilbestrol dipropionate over a

14 week period produced 8 046 lb of milk and 383 lb of butterfat in 305 days

The implantation of progesterone and diethylstilbestrol pellets initially appeared to be an effective method of inducing lactation in heifers and cows that failed to conceive (Meites *et al*, 1951) Two Guernsey heifers implanted with progesterone and diethylstilbestrol pellets produced 331 and 323 lb of butterfat in 306 days Two Holstein Friesian cows received 3 gm of progesterone and 100 mg of diethylstilbestrol, followed 90 days later by an additional implant of 1.5 gm of diethylstilbestrol On the 120th day the nonabsorbed pellets were removed, and milking was begun One cow reached a daily peak of 80 lb, and the second cow attained a maximum daily yield of 45 lb Results obtained at the New Jersey Agricultural Experiment Station from pellet implantation, however, have been variable indeed, probably due to pellet encapsulation In addition favorable responses have not been obtained in the goat (Cowie *et al*, 1952) The milk yields of animals receiving estrogen and those receiving estrogen and progesterone did not differ significantly In general, the hormonally induced milk yields were below the expected yields had the animals come into lactation after kidding

Although estrogens will induce lactation in nonpregnant heifers, they will not increase the production of lactating cows As a matter of fact, the administration of either a massive dose or repeated injections of a smaller amount of an estrogen will decrease milk production Estrogens should not be used on a heifer to bring her into milk production if one desires to get the animal with calf at a later date Indications are that continued estrogenic treatment may cause cystic ovaries One should not inject an estrogen into a pregnant animal unless it is desirable to terminate pregnancy It would appear that more consistent results must be obtained before one is justified in using hormonal therapy for lactational purposes

**Sterile Bulls** Few attempts have been made to determine the influence of hormones on the breeding capacity of bulls Bulls either may be lacking in sex drive or may be producing spermatozoa in capable of fertilizing ova In either case if the bull is in heavy service he should be withdrawn from service and given a rest of two or three months

Before resorting to hormonal therapy, it is well to recall that there are factors other than hormonal ones that may influence the sex drive

of a bull. It is only when extrahormonal factors require no further consideration that one is justified in resorting to hormonal therapy in mature bulls. Therapy should not be used in young bulls, since the lack of sex drive or low fertility may be an expression of an inherited weakness; and if therapy were successful, it would propagate the weakness.

Testosterone has the ability to restore the sex drive of an animal, but it is not known what any given dosage may do to spermatogenesis. If repeated injections are not made, however, it is doubtful whether there would be any harmful influence on spermatogenesis. The injection of a gonadotrophin, either chorionic gonadotrophin or PMS, would stimulate the interstitial cells of the testes to secrete androgen, and this in turn would augment sex drive.

A limited amount of experimentation indicates the possibility of increasing the sex drive of bulls by the feeding of thyroprotein.

Since the injection of FSH into animals from which the pituitary gland has been removed will stimulate spermatogenesis, it might be expected that similar injections would be beneficial in bulls producing semen with a low concentration of spermatozoa. Experimental results are too meager, however, to enable one to make a statement on the possibilities of increasing spermatozoa production in bulls by the injection of a gonadotrophin.

#### HORMONAL THERAPY IN SHEEP

Sheep have a definite breeding season. It begins in the latter part of August or the first part of September, and, in the absence of pregnancy, estrus will recur about every 16 days until December or January (McKenzie and Terrill, 1937). The period from February to August, the time of sexual inactivity, is referred to as the anestrus period. Since sheep have an anestrus period, they have been used rather extensively to determine the possibility of augmenting fertility by hormonal administration. Such work was given impetus by the discovery of Cole and Hart (1930) of a potent gonadotrophin in the blood of pregnant mares (PMS). It was soon shown that PMS would stimulate the quiescent ovary of the anestrus ewe to ovulate and that under certain conditions this was accompanied by estrus, mating, and conception (Cole and Miller, 1933).

Subsequent work showed varying degrees of success with PMS.

Perhaps this should have been anticipated, since there were a number of variable factors such as (1) breed of sheep, (2) nutritional state, (3) time of injection, that is, in early or late anestrus, and (4) number of injections

At the New Jersey Agricultural Experiment Station (Van der Noot *et al*, 1946), 177 anestrus ewes received either one or two injections of PMS (250 to 350 rat units). Of these ewes, 136 came into estrus and mated, 22 following a single injection and 114 following a second injection, which was usually made 16 days after the initial injection. Seventy ewes lambed, 5 of which had received one injection and 65 of which had received two injections.

Treatment of ewes with progesterone followed by an injection of PMS, will induce estrus and ovulation. If these animals are mated, the majority will lamb, however, the number of injections required is probably too great for practical application.

There is a marked drop in the potential reproductive capacity of rams following the onset of hot weather (McKenzie and Berliner, 1937), and if they are to have the desired sex drive it is necessary to inject them with a gonadotrophin (O'Neal 1938). Hence, rams should be injected with 500 rat units of PMS about one week before the ewes are to be injected. When the spermatogenic function of the testis is subnormal in hot weather this can be corrected by the feeding of thyroprotein.

#### HORMONAL THERAPY IN GOATS

Since the goat is a seasonal breeder it is difficult to maintain a uniform milk supply throughout the year. The injection of a gonadotrophin during the anestrus period offers some possibility of inducing estrus and conception. In nonlactating goats 200 rat units of PMS should be injected and if estrus does not occur 400 rat units should be injected 20 days later. Larger doses (600 rat units) appear to be needed for lactating does (Phillips *et al* 1943).

As an alternative to the use of gonadotrophin there exists the possibility of initiating lactation by estrogen treatment. In such instances after lactation is initiated it will be maintained by the act of milking. Experimental work (Folley *et al*, 1940; Lewis and Turner, 1942) indicates that this can be accomplished with varying degrees of success. Satisfactory results have been obtained at the New Jersey



Agricultural Experiment Station by making eight weekly subcutaneous injections of 5 mg. of estradiol dipropionate.

#### HORMONAL THERAPY IN MARES

One of the most fertile fields for research in hormonal therapy for farm animals would appear to be a study of the effectiveness of various hormones in precipitating ovulation at a definite time of the estrous period in the mare. The time of ovulation is extremely variable when computed from the first day of estrus, and this undoubtedly accounts for a part of the difficulty in getting some mares in foal.

Mirskaya and Petropavlovsky (1938) injected subcutaneously 500 mouse units of chorionic gonadotrophin into 68 mares reacting to the teaser and having a follicle one centimeter or larger in diameter. A similar injection was made eight to twelve hours later. Ovulation was induced in 68 mares in 30 to 48 hours after the injection, and 81 per cent of the mares foaled. An average of 1.4 services per mare was required. Ovulation was noted in estrous mares 20 to 40 hours after the intravenous injection of 1,000 to 2,000 rat units of chorionic gonadotrophin (Day, 1939).

PMS appears to be effective in the induction of estrus in mares. Camron (1942) reported estrus in nine mares, previously exhibiting dormant cycles, following the subcutaneous injection of a single dose of 1,000 international units.

#### GENERAL CONSIDERATION

Since many of the hormones are of a protein nature, it should be pointed out that anaphylaxis is at least theoretically possible following their administration. Epinephrine is effective in the treatment of anaphylaxis.

Repeated injections of a hormone of protein nature may result in the formation of antibodies. If antibody formation occurs, repeated injections may not only fail to obtain an increase in response, but may actually result in a decreased response.

In resorting to hormonal therapy, select the hormone that is most likely to give the desired response. For example, in the use of an estrogen it may be desirable to produce an immediate action and

one of short duration. If such is the case, then a nonesterified estrogen such as stilbestrol should be used. When a prolonged action is desirable, as in the initiation of lactation, an esterified estrogen (estradiol dipropionate or stilbestrol dipropionate) should be selected.

## ENDOCRINE PRODUCTS

### ESTROGENS

*Di Ovocylin* ( $\beta$ -estradiol dipropionate), Ciba Pharmaceutical Products, Inc., Lafayette Park, Summit, New Jersey.

*Theelin*, a crystalline estrogenic substance obtained from pregnancy urine, Parke, Davis & Company, Detroit 32, Michigan.

*Stilronate* (diethylstilbestrol dipropionate) Abbott Laboratories, North Chicago, Illinois.

*Stilbestrol* (diethylstilbestrol), Jensen Salsbery Laboratories, Inc., Kansas City 10, Missouri.

*Diethylstilbestrol Winthrop*, Winthrop Chemical Company, Inc., New York 13, New York.

*Stilbestrol Solution* (diethylstilbestrol), Norden Laboratories, Lincoln, Nebraska.

### ANDROGENS

*Perandren* (testosterone propionate), Ciba Pharmaceutical Products, Inc., Lafayette Park, Summit, New Jersey.

### GONADOTROPHINS

A From the serum of pregnant mares (PMS)

*Gonadin*, Cutter Laboratories, Berkeley, California.

*Gonadogen*, The Upjohn Company, Kalamazoo, Michigan.

B From the urine of pregnant women (chorionic gonadotrophin).

*Follutein Squibb*, Division of Veterinary and Animal Feeding Products, E. R. Squibb & Sons, New York, New York.

*Antuitrin S*, Parke, Davis & Company, Detroit 32, Michigan.

*Korotrin*, Winthrop Chemical Company, New York 13, New York.

C From the anterior lobe of the pituitary gland

*Vetrophin*, prepared from acetone-desiccated sheep pituitary powder, Abbott Laboratories, North Chicago, Illinois.

*PITUITRIN*—From the posterior lobe of the pituitary gland

*Posterior Pituitary Extract*, Jensen Salsbery Laboratories, Inc., Kansas City 10, Missouri.

*Posterior Pituitary Injection*, Norden Laboratories, Lincoln, Nebraska.

**EPINEPHRINE**—The hormone of the medulla of the adrenal glands.  
*Epinephrine Hydrochloride*, Norden Laboratories, Lincoln, Nebraska.  
*Epinephrine Hydrochloride Solution*, Jensen-Salsbery Laboratories, Inc.,  
 Kansas City 10, Missouri.

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## General Information

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This chapter deals with general procedures that apply in greater or lesser degree to most classes of farm livestock. Details of the successful methods practiced with each class are given in later chapters.

### SEMEN COLLECTION

Several methods have been contrived for collecting semen from farm animals, but most of them have been abandoned in favor of the use of the artificial vagina. However, since some of the older devices are occasionally employed, a brief discussion of each is included.

*The Artificial Vagina* The invention of this contrivance marked a new milestone in the practice of artificial insemination. Without it, large scale breeding by mechanical means would hardly have developed to its present encouraging status. It was evolved by scientists in Italy and the Soviet Union and found to be highly practical under both laboratory and field conditions. In 1936 the first Danish artificial breeding association for cattle adopted the Russian models and admired their efficiency, because, as A. F. Larsen, its first inseminator, said, "they make possible the collection of a clean, normal ejaculation of semen very quickly." In recent years, artificial vaginas for practically all classes of farm animals have become available commercially. The cylinder part can be made not only of rubber but of tin, iron, wood, porcelain, or other strong material.

One apparatus for cattle is a heavy rubber cylinder  $16\frac{1}{2}$  inches long and  $2\frac{1}{2}$  inches in diameter, fitted with a thin inner rubber sleeve or tube, the ends of which are turned back over the ends of the cylinder and held with heavy rubber bands. A water-tight space is thus formed between the inner and outer walls. Near one end of the cylinder is a hole to admit warm water. In some models this opening is covered by the turned-back end of the liner and the heavy rubber band to prevent the escape of water except under violent pressure, whereas others contain a valve for introducing the warm water and a screw cap to prevent leakage. To complete the assembly, the small end of a rubber cone, or funnel-shaped piece of rubber, is slipped over a sterile, graduated glass test tube; and then the large end, or base of the cone, is fitted over the end of the vagina, with no opening for water (see Figure 16). A small vent (f) is sometimes provided in the cone to prevent ballooning.

The amount of water injected between the sleeve and cylinder should be sufficient to distend the sleeve and create some pressure, thus simulating the natural vagina. Water is usually put in at  $125^{\circ}$  to  $160^{\circ}$  F., depending on the

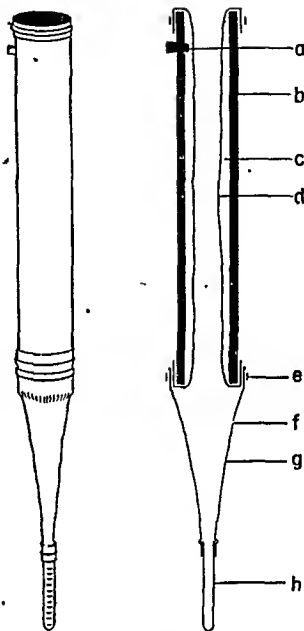


Figure 16. Oanish type of artificial vagina for cattle, after which, most of those used in the United States have been modeled. a. Opening to admit warm water. b. Heavy rubber cylinder to afford rigidity. c. Space for warm water. d. Inner liner of rubber (tube). e. Heavy rubber band to hold end of folded-back rubber liner and to fasten rubber collector cone. f. Air vent to prevent ballooning. g. Collector cone. h. Graduated glass collector tube.

surrounding temperature With a sterile glass rod, a thin coat of sterile lubricating jelly is smeared over the surface of the inner lining at the entering end, although a few operators use no lubricant Do not use petroleum jelly or oil for lubricating rubber Lubricate sparingly to prevent semen contamination For most males the correct temperature of the inside of the vagina at the moment of collection is 105° to 115° F

Most males will respond readily to an artificial vagina if proper precaution is taken to have the water at the right temperature In this connection the desire of certain males must be studied Some of them prefer a higher temperature than others Extremes of temperature especially heat, must be avoided If the male is shocked or perhaps injured by a hot apparatus, he may thereafter refuse to mount for an ejaculation

The advantages of the artificial vagina are Practically all of a normal ejaculate is obtained, the semen is clean and free from extraneous secretions the exact amount of the ejaculate is apparent in the graduated test tube, and the viability of the sperm averages higher than when the collection is made by other methods Limitations of this way of making collections are that a few males refuse to serve the vagina, or injuries and infirmities prevent normal copulation essential to the use of the artificial vagina

*Mechanical Manipulation* The farm animals from which semen has been collected successfully by this method are cattle and fowls Miller and Evans (1934) of the United States Department of Agriculture developed it with bulls by massaging their accessory genital organs principally the ampullae of the ductus deferens

The single advantage of the method is that some semen can often be obtained from valuable bulls which because of being old or crippled are unable to mount a cow for natural service or to ejaculate into an artificial vagina

*Vaginal Collection* One of the oldest methods of obtaining semen is to recover it from the anterior end of the vagina following natural service, with the aid of either a syringe or a vaginal spoon It is a cheap and rather easy way to get a sample of semen for microscopic examination However, as a procedure in large scale insemination, it is not looked upon with favor, for these reasons Only a small portion of the ejaculate is recovered, it is mixed with vaginal secretions



which are sometimes injurious to the sperm, especially when the semen is to be stored; there is great danger of spreading disease if the female carries infection; and there is some danger of injuring the reproductive tract by the recovery operation.

**Electrical Stimulation.** This method can be adapted to nearly all animals. Several methods have been proposed. One of the simplest introduces a weak alternating current to the sacral and pelvic nerves via electrodes placed in the rectum on a probe or by hand. Electro-ejaculator apparatuses are available commercially. Semen is collected in a purer manner than by digital massage because the electrical stimulation causes erection and protrusion of the penis. A small artificial vagina or rubber funnel with test tube is then used to catch the ejaculated semen. Advantages of electroejaculation are: Males need not be able or desirous of mounting a teaser, that is, males which are crippled, infirm, or with low libido can be used. Bulls which are wild or untrained to the artificial vagina also can be confined and electroejaculated satisfactorily.

An exhaustion test by Hill *et al.* (1956) involved 35 collections of semen (196 c.c.) from one bull by electrical stimulation within 75 minutes, without any apparent ill effects.

**Dummies to Aid Collection.** When semen is collected with the artificial vagina, dummies instead of a quiet female, or one in heat, can sometimes be used to good advantage. These eliminate the bother of having a female on hand at collection time. Most bulls prefer a live "decoy." Many operators are skeptical regarding the practicability of the dummy. Reports indicate, however, that certain bulls, stallions, rams, and boars have been trained to use dummies with a fair degree of success, although the young males will usually work better than the old ones. Strangely, an occasional bull will prefer the dummy to the cow at collection time, and there have been cases of another animal species of similar size being used with success.

In training the male to use the dummy, he should be exposed to females in heat but not allowed to serve. After two or more experiences of this kind, he should be introduced to the dummy at the same location. In this way he is taught to anticipate service when brought to the breeding quarters.

The dummy should be strongly built and securely anchored, and it is recommended that it should resemble to some degree the fe-

male of the species for which it is being substituted. The strongest framework is one made of metal. After its sides and top are well padded, the whole structure should be covered with a skin or canvas. The artificial vagina may be held by an attendant or be fastened underneath the rear to a spring attachment. This latter device affords resiliency and is a protection to the mounting male.

**Collection Suggestions** When collecting is done out of doors in cold weather, provisions must be made to prevent subjecting the sperm to cold shock. This can be done by wrapping the semen tube and funnel in a flannel cover or other protective material and by keeping the tube pressed against the warm casing until the male mounts. Some collectors make use of a vagina that is long enough to permit the tube to be contained within it.

#### EXAMINATION OF SEMEN

Just as the careful farmer scrutinizes his grain seed and tests it for viability before planting, so do the cooperative breeding associations and many stockmen today examine the semen of sires that are used regularly or are being considered for use. They are aware that without fertile sperm the whole program for better breeding breaks down. Although the best test of the fertilizing capacity of a male is his ability to "settle" the females which are inseminated to him, nevertheless it should be recognized that he can "go stale," that is, suffer sperm deterioration very quickly. Young bulls without a breeding history are sometimes found to be sterile. Surprising variation exists in the quantity and quality of the semen obtained from different males, and also from the same male. Consequently, the semen of any male should be examined before it is used.

With the increase in artificial insemination, fertility tests are assuming greater importance. The accurate evaluation of the fertility of sperm at time of collection would be a great boon to breeders, but no wholly satisfactory method of making such evaluation has yet been evolved. Most semen tests have been disappointing in efficiency and correlation to fertility. However, using males of medium or low fertility in the cooperative breeding associations involves added expense for extra trips to make reinseminations, and also delays conception in the herds of the members. Subjecting males to semen and collection tests before buying or leasing them either for associa-

tions or for private use frequently saves a lot of time and expense. A good natural breeding record is not quite sufficient, because the quantity and storage quality of the semen may be unsatisfactory; the animal may be too difficult to handle, and he may have picked up infection by natural breeding.

**Volume.** It is important that sires give liberal amounts of semen so that many inseminations can be made if required. Although there is no sure correlation between amount of ejaculate and fertility, generous-sized collections are usually considered a favorable sign. Large volume, however, is not always accompanied by a high sperm count. In attempting to gauge the semen production of a sire, one should not rely on a single ejaculate but use the averages of several taken at different times. Differences in volume are sometimes due to the vigor of the thrust at the time of collection. There should be no distracting noise or other interference.

Proper preparation before service is necessary for securing a large number of the best-quality sperm. Teasing of the male sexually or allowing "false mounts" is frequently practiced. One to three false mounts usually increases the number of strong sperm secured in an ejaculate from the bull.

Various tests have been devised for appraising samples of semen. The best-known measures of semen quality are motility and concentration of sperm and their metabolic activity and livability.

**Motility of Sperm.** Microscopic examination for motility is highly important, because without active movement it is unlikely that the sperm will reach the ovum or penetrate it. Initial motility is believed to be one of the best evidences of viability of semen and fertility, although it is not necessarily a sign that the sperm possess normal fertilizing powers.

In a drop of normal semen under the microscope a swarming mass of sperm is revealed. As the semen cools, motility grows sluggish and soon stops. Examination should be made in a warm room, free from drafts; and the glass on which the sample drop is placed should be kept warm, about 100° F. The accepted method of preserving the liquid semen of large animals for use over a period of one to a few days is to cool it down to approximately 34° to 40° F., the holding temperature. The object is to stop motility and thus conserve the energy of the sperm. It is especially important to have the glass slide warm when examining a sample of semen taken from

the refrigerator or other cooling unit where the sperm have become inactive. On the other hand, care must be taken not to heat the glass much above body temperature, because sperm are killed at about 115° F.

For routine examinations, magnifications of 100 diameters are satisfactory. Examination should be made immediately after collection and then again several minutes after dilution to test the diluting material. The sample for inspection should be representative of the whole ejaculate.

Motility can be estimated most reliably in samples diluted to a sperm concentration of 10 to 20 million per ml. At this concentration the percentage of progressively motile sperm can be clearly detected.

A system of classifying motility can be helpful. A good method for practical purposes is to grade it from 0 to 5 as follows: No motile sperm, 0, less than 25 per cent motile, but weak and oscillatory, 1 (poor), from 20 to 50 per cent progressively motile but lacking in waves, 2 (fair), from 70 to 85 per cent showing vigorous motion and recurring waves, 4 (very good), more than 80 per cent in vigorous motion, with waves of a billowy nature, 5 (excellent).

Different types of motility have come under the eye of the investigator. Walton describes these as a progressive motion by which the sperm move in a straight line, a rotary motion by which the sperm move in circles with diameters of about their own length, and an oscillatory motion that is convulsive in nature and without progress. Without progressive movement, fertilization is unlikely.

Mammalian sperm possess rectilinear movement by which they can change both their direction and speed when meeting a flow of liquid. Rikmenspoel and van Herpen (1957) observed from cinematographic studies that the orbit of the sperm is normally only slightly curved, there is cell rotation and a tail wave which is three-dimensional. By using the photoelectric method the mean velocity of normally active cells was measured with only a 3 per cent error. In estimating sperm numbers the error was close to 10 per cent.

Lardy and Phillips (1941) reported that motility is sustained chiefly by the glycolytic or fructolytic mechanism. The rate of movement varies with the species, the health of the sperm, and the kind of medium.

The middle piece of the sperm is believed to be the motor part. With loss of the head, the middle piece and tail continue forward.

in a straight line. When the tail is injured, motility may cease or the sperm moves in circles or backwards.

**Color and Concentration.** The color of semen is important because it is an indication of its quality. Normal semen of most species has the appearance of whole milk, its opacity being due almost entirely to the dense mass of sperm. Samples of semen of lesser density are characterized by a more watery appearance, down to the almost transparent type of ejaculate. Investigators early noted that marked cloudiness, due to swirling masses of sperm, was usually associated with high rate of conception.

The range in sperm count per ejaculate of the semen is quite wide, varying at times from 25 million to 1 billion per c.c. for the boar, 30 million to 800 million for the stallion, 500,000 to 60 million for the cock, 300 million to 2 billion for the bull, and 800 million to 4 billion for the ram. For example, good samples of bull semen fluctuate around the 800 million mark.

Those who work with the semen of certain species soon learn to associate color with quality to an appreciable degree. Abnormal color may reflect an unhealthy condition of the genital organs. A yellowish color may indicate the presence of pus or urine, which can usually be detected by the smell. When blood or degenerating tissue is present, the color is pinkish or reddish. Abnormal semen should never be used. Sires giving it should be examined and treated in an effort to remove the cause.

The concentration of sperm varies widely, both in ejaculates of the same male and from male to male of the same species. It is of great value to know the approximate number of sperm in a cubic centimeter of semen, for this is a measure of its quality and determines the rate for diluting it for routine insemination. There are now several methods of calculating sperm count, the oldest of which is the use of the hemocytometer. A newer and more practical device for use at large breeding headquarters is the photoelectric colorimeter.

**Abnormal Sperm.** Making periodic, microscopic examinations for abnormal sperm, bacteria, protozoa, and cells which might indicate a diseased condition of the male may reveal the cause of sterility or poor conception rate. The greater the proportion of normal, healthy sperm, the greater the likelihood of fertility. More information is needed, however, concerning the question of the relationship be-

tween the percentage and type of abnormalities and the fertility of the male

Listed in the order of their usual frequency of occurrence, these are as follows: looped tails, tailless or headless, broken tails, those having pyriform (pear shaped) heads, and those with enlarged middle pieces. Haq (1949) lists the permissible limits of abnormalities of the head, middle piece, tail, and tailless heads as 4, 8, 2, and 6, respectively, or a total of 20 per cent for semen of normal quality. In studies with bulls it was found that those with less than 500 normal sperm per 1,000 had an unsatisfactory breeding record, whereas those with 900 or more usually proved highly fertile.

Sperm may be normal in morphology but wholly nonfertilizing. Factors other than morphology are responsible for duration of the necessary motility. Semen that rates well in motility and viability usually produces good breeding efficiency.

**Longevity of Sperm.** The livability of sperm bears some relationship to their fertilizing powers. The rate of non return to service, as reported by Fryer *et al* (1958), in a trial involving bulls, varied from 5 to 11 percentage units (depending on breed) for each additional day of storage of liquid semen. The first tests for longevity were carried out by noting how long the sperm remained motile at a standard storage temperature of about 40° F. This system usually requires periodic examination of the semen samples for several days. The high temperature test and the methylene blue reduction test are also correlated with longevity. (These are discussed in the chapter on cattle.) There is a lack of agreement among research workers regarding the value and practicability of these two tests. Work by Chang (1946) indicates that a certain period of time in the female tract seems necessary for the sperm to acquire capacity for fertilization.

If semen is to be stored by freezing, the only way to determine satisfactory freezability is to make a test freeze. Semen of some fertile bulls will not survive freezing to an adequate degree.

**Combination Staining.** A combination stain which shows sperm morphology as well as live and dead sperm can be made by using a solution of 1 per cent eosin B, 5 per cent nigrosin, and 2.5 per cent sodium citrate in distilled water. A small speck of semen is mixed with a glass stirring rod with a drop of the stain solution on a clean glass microscope slide. Place another slide on top to spread the

mixture evenly, then separate the slides by sliding apart and dry the smears *instantaneously* over a warming plate.

Examine the sperm at 400 to 900 diameters magnification. Dead sperm will stain bright red, and all sperm are tinted enough to show shape and structure of the cells. Staining semen in a similar manner with a 3 per cent solution of rose bengal stain results in bright staining of all sperm. The rose bengal stain must be rinsed off the slide after drying to allow clear view of the spermatozoa.

#### HANDLING THE SEMEN

The sensitive nature of the sperm necessitates the utmost care in the handling of semen. If a high state of viability is not maintained, the percentage of conceptions will be small or nil. The following precautions should be taken: Protect from extreme heat or cold; prevent contact with water and harmful chemical agents; and avoid exposure to air and direct sunlight as much as possible. Semen should not be shaken. The tube should be full to avoid air space. Mann (1945) said that when sperm in suspension were agitated vigorously in the presence of air, the cytochrome enzyme within the sperm was oxidized.

#### SEMEN DILUTERS

For more than two decades after 1935 much of the research work involving artificial insemination and semen characteristics dealt with diluters. A full report would require hundreds of pages. The chief function of diluters is to expand the volume of semen so that many times the usual number of females can be inseminated. They also provide protective factors for the sperm-buffering compounds to maintain the proper acid-base balance, and nutrient materials and bacteriostatic or bactericidal substances—all of which contribute to improved livability of the sperm in storage.

The most extensive use of diluters is in breeding associations and on ranches and farms where large numbers of animals are in the insemination program. Without the diluters, these programs, involving wide utilization of many of the greatest males of the different species of farm animals, could not exist. More experiments in search of new and better types of diluters are certain to continue. For cows, some operators use dilutions as high as 1 part of semen to 200 parts



Figure 17 Pen sketch of bull sperm showing normal wave formation magnified approximately 318 diameters (Courtesy Erik Blam Royal Veterinary and Agricultural College Copenhagen)

of diluter, although dilutions of 1 to 100 or 1 to 50 are more common

The chief requirements of a good diluter are that it be nontoxic to sperm, simple to prepare, low in cost, favorable to longevity of sperm, and easy to keep, and that it provide the functions listed above

**Egg Yolk-Citrate Diluter** This was devised by Salisbury, Fuller, and Willett (1941) of Cornell University. A 3 per cent sodium citrate



solution ( $\text{Na}_2\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) is made with water distilled over glass. Prepare the egg yolk from fresh eggs after washing them and then rinsing them with 70 per cent alcohol. Allow them to dry before breaking the shell with a sterile knife. Use only the yolk, removing all of the egg white from around it by rolling it on absorbent paper. Puncture the yolk membrane, and allow the yolk to flow into a sterile glass container. Mix together 4 parts by volume of the buffer solution and 1 part of the egg yolk for the completed diluter, based on the work by Swanson (1949). The discovery by Phillips and Lardy (1940) of the protective effect of egg yolk in diluters for bull semen was soon followed by the formulation of many satisfactory egg yolk-buffer diluents. The egg yolk protects the sperm cells against damage during cooling and other unfavorable environmental conditions. Isotonic buffers to maintain a pH of 6.7 to 6.9 can be made from phosphate, citrate, carbonate, and other salts.

**Milk Diluter.** Fresh or pasteurized milk will kill sperm because of enzyme effects. However, if milk or skim milk is heated to 200° F. (nearly boiling) for 10 to 20 minutes, the enzymes are destroyed and the milk makes a compatible semen diluter. Milk has some of the protective factor found in egg yolk, but recent experiments indicate that milk can be improved as a semen diluter by adding to it 10 per cent egg yolk. This diluter is popular with some cattle breeding organizations. It has been found that the addition of glycerol to the diluter also improves the livability of bull sperm.

**Antibiotics in Diluters.** Addition of compatible antibiotics to semen diluters is recommended to control bacterial growth. A combination of streptomycin at 500 to 1,000 mg. per ml. and penicillin at 500 to 1,000 units per ml. is satisfactory. Neomycin and polymyxin have also been used. Several "sulfa" compounds (sulfanilamide, and others) are also useful in this respect. The antibiotics may result in better conception rates when used with semen infected with uterine invaders, such as *Vibrio fetus*. They also aid in maintaining satisfactory sperm viability during several days' storage.

**Reactivation.** In a trial by Rickard *et al.* (1957) two-day-old activated bull semen (liquid) gave a non-return rate of 70.33 per cent, compared with a rate of 60.34 per cent for the control. Two-day-old samples were just as good as one-day-old samples.

## FROZEN SEMEN

Semen of some species, previously kept successfully for a few days, has been frozen and stored for months and years with little or no ill effect. The conception rate in the case of bull sperm is sometimes as high as that obtained with fresh semen. In several instances it has been higher. Frozen semen is used in practically all present day cattle artificial breeding organizations in many countries. In fact, some of the groups operate a 100 per cent frozen semen program. The plan makes possible the maximum use of desirable sires, even for a period after death. Using frozen semen two years old, stored at  $79^{\circ}\text{C}$ , Mixner and Wiggins (1957) obtained the same conception rate of 65.7 that they had gotten when the same hatch of frozen semen was one year old. The method, which involves the freezing of semen to  $-79^{\circ}\text{C}$ , the temperature of dry ice (solid  $\text{CO}_2$ ), and holding it at that point or lower, also includes the employing of glycerol and other material for protection. The process was developed by the British National Institute of Medical Research and the University of Cambridge, England.

## FREEZE DRYING

Based on successful lyophilization for preserving blood plasma, milk, bacteria, viruses, and so on, Albright *et al* (1958) freeze dried bull sperm from five bulls. Between 5 and 10 per cent of the sperm showed progressive mobility after reconstitution. Polge *et al* (1949) had first made similar progress with fowl semen, using the process of high vacuum distillation. The outcome of such experiments cannot be predicted at the present time.

## CONCEPTION RATE

One of the best measures of conception rate is the percentage of females that conceive on first insemination. Certain important factors influence it. In normal mating there are two variables, the fertility of the male and the fertility of the female. The use of artificial insemination introduces a third, the technique of the method. Each factor may be discussed in terms of an efficiency level. In nor-

mal mating it is rare to find a state of affairs in which male and female operate constantly at the 100 per cent level; but even if the figure in each case is 80 per cent, the combined effect is a conception rate of 64 per cent ( $80 \times 80$ ). Good technique will maintain this figure, but for each drop in efficiency there is a lowering in the rate. It is possible to work out many combinations of these three variables, to see that each constitutes a limiting factor and that a low level of efficiency in any one may be more detrimental to the conception rate than a moderately good level in all. There are certain factors that have a direct bearing on the results obtained. Among these are the insemination at the optimum time during the heat period.

#### ESTROUS CYCLE, ESTRUS, AND OVULATION

The estrous cycle is the time between heat periods; estrus is the heat period itself; and ovulation is the shedding of the egg (or eggs) by the ovary preparatory to the egg's movement down the Fallopian tube. It is to the interest of every livestock owner to possess a thorough understanding of these phenomena of nature. The conception rate, the timing of the season or month of birth of the young, and the economical use of the reproducing female depend on a good working knowledge of them.

The relation that heat bears to ovulation is very important. Insemination should precede the latter by a few hours or should coincide with it. Considerable variation occurs within the same species in the duration of heat and the time of ovulation after heat. The exact time of the beginning of heat is often impossible to determine, but practice has shown that for at least the bovine stock, inseminations made during the last half of the period have a better chance of success than those made earlier.

Because of the impossibility of forecasting the length of the heat period, two or more inseminations are sometimes made in animals having long heat periods. Some horse breeding authorities advise inseminating a mare on the second and fifth days, or even on the fourth, sixth, and eighth days after the start of heat, while others practice breeding a second time if she is still in heat the third day after the first insemination.

Russian sheep stations have increased their lambing percentages markedly by inseminating their ewes several times in the same heat.

With cows, however two inseminations per period have not produced impressive experimental results

#### INSEMINATION

**Deposition of Semen** There seems to be general agreement that the best rate of conception is obtained when the semen is placed in the cervix or injected into the uterus. Walton, a pioneer in this field, noted that in sheep experiments the introduction of 0.5 to 1.0 c.c. of semen into the cervix of the ewe gave him 66 per cent conception, as contrasted with 33 per cent when the same amount was placed in the vagina. The most desirable region of deposition has not yet been fully established for all of the farm animals. In the case of the sow, there seems to be no doubt that the uterus should be the place of deposition, as it is in copulation. Inseminators of fowl place the semen in the oviduct.

**Quantity of Semen** Is there an optimum number of sperm to be used in the artificial insemination of different species? Important facts pertaining to this problem have been established in recent years. For centuries, biologists have known that nature is extravagant in anticipation of reproduction and that there is much wastage of seed in both the plant and the animal kingdoms. The discovery that relatively small quantities of viable semen can effect conception in most animals has been mainly responsible for the recent growth of artificial insemination.

The matter for concern is the required number of viable, healthy sperm per implantation rather than the amount of semen to use. This explains why quick tests for gauging the approximate number of sperm per cubic centimeter are so important. For cows some technicians advise a minimum of 10 to 15 million sperm per insemination. Reports of Russian studies indicate that the use of quantities of semen in excess of 2 c.c. per insemination did not increase the rate of conception in cattle. For example, if the collection averages 1 billion sperm per c.c. and the dilution is at the rate of 1 portion of semen to 40 of diluter, the approximate number of sperm per c.c. of the diluted semen will be 24 million.

## INSEMINATION EQUIPMENT

The equipment needed for the collection and preservation of semen and the insemination of each species, either in a breeding association or on the farm or ranch, should include most of the following:

1. Microscope with glass slides and cover slips.
2. Artificial vagina for species worked with.
3. Lubricant for preparation of artificial vagina.
4. Thermometer for taking temperature of artificial vagina and refrigerating container.
5. Sterile test tubes, some graduated for semen collection.
6. Beakers, some graduated 100-c.c. and 500-c.c. sizes, for preparing solutions.
7. Labels for marking tubes and vials.
8. Rubber stoppers for test tubes and bottles.
9. Refrigerator.
10. Test tube rack.
11. Syringes or bulbs and inseminating tubes (catheters) adapted to species.
12. Combination semen and equipment carrying case (refrigerated).
13. Rubber gloves and sleeve.
14. Talcum powder in shaker can.
15. Rolls of sterile absorbent cotton and paper towels.
16. Coveralls and rubber boots.
17. Pail large enough to enable washing and disinfecting boots between farms or herds.
18. Suitable powder and brushes for washing equipment.
19. Physiological saline solution.
20. Distilled water for rinsing glassware.
21. Enameled pan, 12 by 18 by 3½ inches, for washing equipment and for holding it afterward.
22. Alcohol for sterilizing rubber equipment.
23. Indelible pencil for signing breeding receipts.

Other special apparatus is mentioned in the discussions dealing with the different farm animals. Two important pieces of equipment used by the larger commercial breeding associations are the electric water heater and the electric drying oven. Much of the equipment formerly made only of glass is now available in nontoxic plastic.

**Cleaning the Equipment.** Since sperm are quickly killed or weakened by toxic substances, it is very important that all equipment be scrupulously cleaned, sterilized, and left sterile until needed.

All new apparatus should be well washed before use so as to remove any dirt, grease, and chemical matter. It should also be sterilized. After each use, too, every piece of equipment must likewise be thoroughly cleaned and sterilized. Glass inseminating tubes can best be cleansed by drawing some of the cleaning solution through them several times with a rubber bulb or syringe. The syringe should be taken apart and scrubbed with a brush. After use of the artificial vagina, the rubber liner and cone should be washed at once with warm water, then rinsed first in distilled water and then in alcohol and dried. The rubber parts are distended by means of clothespins. Plastic tubes and bulbs are used only once, so they are not washed.

**Sterilizing the Equipment.** Some technicians disinfect all washed equipment, including the rubber, by letting it stand five to ten minutes in a 65 to 70 per cent solution of ethyl or isopropyl alcohol. If this is done, and it must be used before dry, the alcohol should be washed off by rinsing in sterilized buffer solution or semen diluter. More common and economical methods of sterilization are boiling in water or the use of dry heat. Practical steps should be taken in the following order. Boil glass syringes, glass inseminating tubes, test tubes, and glass speculums for 15 minutes. With the aid of forceps dip the rubber tubing and stoppers in the boiling water and remove at once. After shaking off the water, place the rubber parts in a drying cabinet, or dry between towels. Glass equipment may also be sterilized by placing it in a metal pan and baking it in an oven at 250° to 350° F. for an hour.

**Inorganic Salts for Cleaning Equipment.\*** The synthetic organic detergents, whose wetting properties have made them so useful for washing dairy equipment, possess bactericidal and bacteriostatic properties. They are characterized by very high adsorption coefficients; even when they are used in dilute solution, the surfaces of the equipment contacted by their solutions will show a comparatively high concentration of them. The simple construction of sperm cells makes it quite logical that those wetting agents which exhibit definite bactericidal properties will adversely affect sperm. Because

\* This information was supplied by the New Jersey Dairy Laboratories, New Brunswick, New Jersey.

of these factors, the synthetic organic detergent should not be used in the washing and sterilizing of artificial insemination equipment.

On the other hand, sodium hexa-meta-phosphate (Calgon, and others) and tetra-sodium pyro-phosphate are two inorganic salts whose calcium sequestration properties are as great as those of the organic detergents, although they are inferior to many of the organic compounds in their wetting abilities. Their solutions are not bactericidal or bacteriostatic (they decompose ultimately to simpler sodium phosphates, and these are commonly used to buffer nutrient solutions). Ample water rinsing is adequate. Their lack of bacteriostatic effect eliminates the need for removal of traces. The phosphates are not corrosive to glass or to rubber and are as non-corrosive to metalware as most of the wetting agents. The following procedure for washing artificial inseminating glass, rubber, and metalware will prove satisfactory.

1. Directly after using, rinse and brush all glass, metal, and rubber equipment surfaces with a lukewarm water solution of 0.2 to 0.3 per cent sodium hexa-meta-phosphate or 0.3 to 0.5 per cent of tetra-sodium pyro-phosphate. Applying some of the dry phosphate on moist brush bristles will greatly accelerate cleaning. Small equipment—syringes, inseminating rods, and so on—which cannot be brushed should be soaked for a few minutes, then have the solution pushed through so that the speed of flow will increase washing efficiency. Higher velocities aid this erosion.

2. Rinse with lukewarm water.

3. Drain; rinse all parts with a small amount of 70 per cent ethyl or isopropyl alcohol. Permit to dry in a dust-free, ventilated storage space.

Seventy per cent alcohol is a very satisfactory sterilizing agent for fully cleaned equipment because it leaves no residue. If the equipment is moist after washing, the potency of 70 per cent alcohol will not be reduced if the surfaces have been drained.

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interest and vigor In the females, the estrous cycle stopped, and ovulation failed

When Mann and Walton (1953) studied the effects of a 23-week period of underfeeding of bulls they noted little or no change in the volume and density of the semen or the mobility and morphology of the sperm However, they did find that the secretory function of the accessory glands was much affected by the lowered nutrition The decreases in the concentration of citric acid and fructose were 60 and 30 per cent, respectively, of the original levels But in the recovery period, the values for both of these returned slowly to normal

Mann and Rowson (1956) detected sperm in the semen of a bull calf on a high plane of nutrition at nine months of age In the semen of this calf's identical twin, raised on a low plane, they appeared one month later The activity of the latter animal's accessory glands, as reflected by the presence of fructose and citric acid in the semen, was delayed four months Animals considered to be very intelligently fed, however, are not always good breeders In fact, too liberal feeding frequently lowers breeding efficiency McKenzie and Phillips (1937) noted that highly conditioned, heavily fleeced rams sometimes yielded many abnormal sperm and were of low fertility and occasionally sterile One show winner had 75.9 per cent of abnormal sperm and failed to impregnate 34 ewes After shearing and reduction in weight, the percentage of abnormal sperm in his semen fell to 15.8 per cent But he was soon fitted again for the show circuit, and at the end of four months his sperm showed 69.2 per cent abnormalities However, heavy feeding does not always prove injurious to animals Certain ones naturally gain weight more quickly than others and continue to do surprisingly well as breeders for a considerable length of time, as reported by Hart and Miller (1937)

The fertility of sperm cells was not seriously impaired by uncommonly high or low feed and nutrient intakes during early growth and development, as reported by Bratton (1957) But the onset of the reproductive function was delayed if feed or nutrient intake was too low for normal rates of growth and development In a study of pasture versus dry lot feeding for bulls three feeding regimes were used by Branton *et al* (1953) grain and pasture alone grain and hay alone grain and pasture and hay Sexual behavior, semen production and fertility were the same for all groups



**Protein.** It is generally recognized that males in heavy service require a liberal amount of protein. Hansen Larsen *et al.* (1944) reported a marked improvement in bull fertility when animal protein comprised 70 per cent of high protein intake. On the contrary, Webster (1932) observed that a high incidence of sterility seemed to be associated with morphologically abnormal sperm ejaculated by New Zealand bulls which grazed pastures in which the crude protein content of the grass ran as high as 35 per cent of the dry matter. Branton *et al.* (1947) reported that the fertility of bulls was not significantly affected by concentrate mixtures having 12, 16, and 20 per cent protein. The 20 per cent concentrate resulted in a greater sperm concentration, but a lower volume of ejaculate, less motility, and less sperm per ejaculate. The Russian worker Smirnov-Ugrjumov (1937) obtained more and better semen from bulls that had in their ration such animal protein carriers as bone meal or fish meal, or skim milk or blood meal, instead of ground oats and other concentrates. The experiments by Cunningham and Hopkirk (1935) revealed that rats given a diet low in lysine had underdeveloped testicles. When various proteins (fluid skim milk, dried skim milk, and vegetable protein) were fed to calves from the ages of three weeks through ninety weeks, the source of the protein did not seem to make any significant difference in the age or size at which viable sperm were first collected from the bulls (Flipse *et al.*, 1956).

Four sets of identical twin bull calves were used in a trial by Flipse (1957) to compare an experimental ration of corn silage and hay with a control ration of concentrate and hay. The twins were from thirteen to one hundred and twelve weeks of age, inclusive. Total digestible nutrients (T.D.N.) consumption was maintained at the level recommended by the National Research Council. Digestible protein intake was lower for the bulls fed silage than for those fed concentrate. Growth, attainment of sexual maturity, and quantity and quality (total number and concentration of sperm ejaculated, initial motility, and storage possibilities) of semen produced were similar in the two groups. Corn silage is a good source of nutrients for maintaining fertility in bulls. The theory that heavy feeding of grass silage tends to impair the fertility of bulls was exploded by Flipse and Almquist (1957) in a trial with 42 animals. There was no appreciable reduction in motility or concentration of sperm

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## Factors Influencing the Quality and Quantity of Semen

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Not all of the factors that can affect the conception rate of livestock are known. Some of those once thought to be significant are now deemed unimportant. Reference is made in this chapter only to those most frequently discussed in relation to the quantity and quality of semen.

### NUTRITION

Too little information is available regarding the nutritive factors required for normal reproductive functions in farm animals. Much of what exists is contradictory and therefore misleading. But the considerable research now under way at several experiment stations should furnish highly helpful facts.

Faulty nutrition has long been recognized as having an injurious effect upon the male reproductive system of certain species. In terms of energy intake, Evans *et al.* (1922) found that a decrease of 30 per cent, or less, in the amount the rat consumed on a self-fed ration would adversely affect reproduction. When underfeeding was drastic enough to retard markedly the growth of the immature male animals or cause loss of weight of the mature, the testes revealed atrophy, there was loss of mobile sperm, and the rat lacked sex

among the bulls which ate large amounts of silage, and the feed cost was reduced

**Minerals** The minerals most likely to be lacking in the feeds available in the leading livestock areas are calcium and phosphorus. A good grade of legume or mixed hay furnishes ample calcium, and when protein concentrates are fed, a phosphorus deficiency is unlikely. Where these two elements are lacking, however, they can be economically provided in dicalcium phosphate.

Strauch and Brunner (1956) reported that on 57 farms in Germany where the cattle had serious reproductive disorders, an analysis of the soil and hay samples established a direct relationship between the phosphorus content of the fodder, the calving index, and the insemination index. Reid (1949) says that reproduction is not usually affected until the symptoms of phosphorus deficiency appear, and that many cases of cattle sterility in phosphorus deficient areas may not be due, entirely or necessarily, to the lack of this element. The low phosphorus in the forage is accompanied by a low protein level, and other dietary deficiencies may be involved.

In addition to iodine, the other trace elements sometimes, though seldom, needed as supplements are iron, cobalt, and copper. Certain soils and the crops from them may be deficient in these minerals. Hence the mineral mixtures sold in those areas usually contain the necessary materials. The feeding of salt to livestock should never be neglected.

According to Albrecht there is a close relationship between animal fertility and soil fertility. He reports that pregnancy disease in sheep was aggravated by soil deficiencies; that poor soils produce poor plants, especially in regard to content of minerals, and that general good health for man and his animals is dependent in a large measure on fertile soil.

**Vitamins** Vitamins A, C and D seem to be the most important ones related to breeding, but the exact vitamin requirements for service bulls are as yet unknown. It is believed, however, that a deficiency in this phase of nutrition seldom occurs when proper attention is paid to feeding a ration that is basically high-quality roughage. Hodgson *et al* (1946) caused typical vitamin A deficiency symptoms in 12 dairy bulls by adjusting the intake of this vitamin. The semen was of low density with a high percentage of abnormal

sperm and a high pH, and it did not store well. Seven of the bulls remained fertile, but with a low conception rate.

Based on a study of the relation of carotene levels to fertility in dairy bulls, Jones *et al.* (1946) report that 15 to 35  $\mu$ g. of carotene daily per kg. of body weight seem to provide enough vitamin A for Jersey bulls (the breed used in the experiment) to retain their fertility. Two of the five bulls in the experiment became blind during early growth but exhibited normal male behavior at breeding age.

Gunn *et al.* (1942) of Australia found that the fertility of rams was rapidly reduced when they were deprived of green feed for several months during prolonged droughts. After seminal degeneration occurred, these workers induced recovery from vitamin A deficiency with a daily diet consisting of 0.3 lb. bran, 0.4 lb. oats, 0.3 lb. linseed cake, 1.1 lb. good whcaten chaff, and 0.7 lb. legume (lucerne) hay. The condition was also prevented, improved, or cured by feeding a mixed vitamin concentrate composed of a concentrate of vitamin A (containing 435 international units of vitamin D), liver meal, molasses, carrots, and green grass. Within two to four and one half months deficient diets caused seminal degeneration, and two to three months were required to bring recovery after the supplements were begun.

Properly cured hay and silage, when fed in liberal amounts, usually supply sufficient vitamins A and D to bulls, rams, and stallions, but stored hay loses much of its vitamin A rather rapidly. Young pasture grasses are high in carotene, which in turn is changed into vitamin A by the animal. Consequently, males which have been given plenty of green feed during the growing months go into the winter with a large reserve of vitamin A stored in their bodies.

There is no present evidence to suggest that the larger animals ever suffer from a serious vitamin E deficiency. This vitamin is distributed widely in natural feeds. An experiment in which Salisbury (1944) fed ten bulls wheat germ oil for a year failed to reveal any improvement in their fertility.

In conclusion, it can be said that although malnutrition may affect the reproductive performance of male livestock in various ways, a deficiency of a single element is very seldom noted under conditions usually prevailing on farms or at headquarters of breed-

ing organizations It has not yet been proved that a ration which seems suitable, both in quality and quantity, for normal growth and, apparently, good health is also the best for maintaining normal reproductive powers But it is encouraging to reflect, as stated by Reid (1949), that "livestock in general do not experience specific, nutritional deficiencies that perceptibly influence reproduction under good feeding conditions"

#### TEMPERATURE AND SEASON

The effect that extremes of atmospheric temperature can have on the reproductive processes of the male is highly important It has been demonstrated that extra heat applied to the scrotum can affect both spermatogenesis and mature sperm and that extreme cold causes physiologically subnormal sperm For proper functioning in mammals, the testes require a lower-than body temperature The exposed position of the scrotum and its tunica dartos muscle, a fibrous layer situated immediately beneath the skin of the scrotum, is chiefly responsible for maintaining the optimum temperature for the testes Increased warmth causes this muscle to relax, automatically widening the spaces between the testes and the body, a fall in the atmosphere temperature induces contraction of the muscle Russian workers noted a drop in the quality of sperm of rams kept in a shed where the temperature ranged from 80° to 90° F Males of certain species and breeds are poorly adapted to losing heat by sweating, a factor influencing the quality and quantity of semen

Closely associated with atmospheric temperature as a factor that can influence quality and quantity of the semen are the seasons Gunn *et al* (1942) reported that hot weather caused seminal degeneration in rams, and that recovery usually occurred in the cooler season In some cases it was considered that deficient nutrition was an allied factor in the trouble So positive were the results of their study that they developed a method of predicting probable periods of highest fertility from information on temperatures and pasture conditions They foretold with considerable accuracy the quality of semen likely to be produced by wholly pasture fed rams at any given time for different areas When supplementary feeds were given, the quantity and quality of the semen were influenced only by the heat

Dutt and Hamm (1957) studied rams during the month of January. Controls were maintained conventionally. Experimental rams, sheared and unsheared, were placed in a 90° F. temperature room for one week. Volume of semen was little affected by treatment. Motility of sperm cells and sperm cell concentration decreased, and the number of abnormal sperm cells increased as the result of the elevated temperature. The unsheared rams were much more affected than were the sheared rams. All animals returned to normal after treatment. This work shows the importance of shearing in helping to prevent lowering of semen quality during hot weather.

Fertility of sheared rams was significantly higher than that of unsheared rams without their being a significant difference in semen quality, as indicated by a report of Hulet *et al.* (1956). Casady *et al.* (1953) concluded that under chamber conditions, spermatogenesis in young bulls may be impaired when the animal is continuously exposed to an 85° F. temperature for periods exceeding five weeks.

The work done by McKenzie and Phillips (1937) in Missouri revealed more frequent completed copulations and better sperm for Shropshire rams from October to January and for Hampshires from August to January. The rams continued to manifest mating desire throughout the year. Green (1940) noted a decline in sperm concentration of Shropshire ram semen from 3.5 billion per c.c. in May to 1.36 billion in August, but by December it had risen to 3.41 billion. The month for highest number of normal sperm was November, and the lowest was mid-July.

The results of studies of the relation between bull semen quality and climatic factors in different parts of the United States are at variance. Erb *et al.* (1942) in Indiana obtained semen of lowest quantity and quality in July, August, and September and of highest quantity and quality in April, May, and June. The bulls were managed in the same way throughout the year of the trial. The differences were attributed mainly to temperature and relative humidity, the chief factors associated with change of season. In a study of the seasonal fertility of aged bulls in Missouri, Swanson and Herman (1941) reported that initial motility and useful viability were lower in winter weather than in spring and summer—conditions believed to be due to the effect of adverse winter weather on the health and sexual activity of the bulls. Working in Arizona with beef bulls, Lasley and Bogart (1943) found that they yielded the best sperm

from May to September Their findings are in agreement with Anderson (1945), who reported that "there appears to be a basic seasonal rhythm in bull semen associated with climatic factors in Kenya, warmer conditions causing stimulation, and vice versa In a study of 71,098 cattle artificial insemination services in Kansas, where it was felt that climatic conditions were more nearly typical of the southern half of the United States, the highest average non-return rate occurred in the fall season and the lowest in the summer

The specific causes of these fluctuations are not known They are not simply related to maximum, minimum, or mean monthly temperatures It is conceivable that the humidity prevailing in certain areas, some of them desert like and of high altitude, may help to account for lack of agreement in the results of some of the studies reported The degree of acclimatization as well as the species and breed can also be listed as influencing semen production

#### FREQUENCY OF USE OF MALE

There is much individual variation in the number of times that males can be used without impairing their fertility What appears to be normal performance for one will be sex strain for another. Twenty years of artificial breeding of dairy cattle in the United states prove it is sound economy to use an ample number of bulls at the studs

The sperm producing powers of healthy males are not quickly exhausted, but they should never be abused When Weatherby *et al* (1940) took ejaculates from a bull once daily for 57 days, the volume of semen was well maintained, but sperm concentration varied from 2,179 million sperm per c c in the fourth week to none at the beginning of the ninth week The longevity of the sperm increased slightly until the fifth week and then declined rapidly In another test with a virile three year old bull, two ejaculates were collected every third day for 11½ months The volume decreased, but the concentration and longevity of the sperm remained satisfactorily constant.

The rate of sperm production in many bulls is probably greater than has been previously recognized Using six bulls, Hale *et al* (1953) made continuous collections until the bull refused to work They discovered that the quantity and quality of semen had returned to pre-exhaustion level for all of the bulls within one week after





Figure 18. A very remarkable proved sire is the Halstein Kanowa King Pasch Neptune, owned and used by the American Breeders Service of Madison, Wisconsin. To his credit, at the age of fifteen, are listed 150,000 first services. His daughters have been producing an annual average of 1,280 lb. more milk than their dams. This achievement typifies artificial insemination's major objective of multiplying the germ plasma of the best proved bulls.

the exhaustion tests. One ejaculate every fourth day or two every eighth day for one year was not detrimental to fertility in trials by Bratton and Foote (1954). Ejaculations collected at the rate of two at eight days apart yielded 60 per cent more motile sperm than one at eight days apart. The trials by VanDemark *et al.* (1956) indicated the potential services of a certain Holstein bull to be as many as 371,058 over a four-year period when ejaculated three times per week.

Baker *et al.* (1955) found that frequent ejaculation, up to three times per week, did not affect anything except libido (which was adversely affected). Boyd and VanDemark (1957), using ten dairy bulls from one and one-half to four years old, conducted a series of partial exhaustion tests (ten consecutive ejaculates within 90 min-

utes) The extremely depleted sperm supply was fully restored after a seven day period Lengthening the interval between exhaustions to 30 days did not result in any more sperm than did the seven-day interval

Table V shows the effects of an exhaustion test—though not extreme—on the semen of a bull when eight ejaculates were col-

TABLE V EFFECT OF FREQUENCY OF COLLECTION ON SEMEN VOLUME, SPERM CONCENTRATION, AND FRUCTOSE CONTENT OF BULL SEMEN

Ejaculate no	Time of collection (min)	Volume of ejaculate (ml)	Sperm concentration (millions)	Fructose content (mg per 100 ml semen)
1	0	4.2	1,664	760
2	10	3.9	680	790
3	18	3.7	254	900
4	28	3.7	648	750
5	38	3.4	135	820
6	45	3.5	342	820
7	55	2.7	390	630
8	63	2.9	98	690

Source: Mann 1954

lected from the same bull within 63 minutes at intervals of seven to ten minutes

The difficulty of exhausting the sex organs of rams was noted by Kuznetsov *et al* (1930) After a total of 42 ejaculates were collected from one ram in nine hours, the sperm concentration was still 100 million per c c The first ejaculate contained 25 times that number Gunn *et al* (1942) reported that rams ejaculated as often as 8 times in 10 days and 17 times in 45 days showed no radical decrease in the duration of motility, percentage of active sperm, or volume of semen, neither did the morphology of the sperm seem to be affected in any way In the experience of Lambert and McKenzie (1940), rams could stand three and in some instances even more matings daily during the breeding season without apparent injury to their breeding powers

Anderson (1945) says "It would appear there is a certain wave-

like progress of sperm from the testis and that differences occur in the rate at which they are pushed forward. The rate in some rams must be so fast that each ejaculate contains a normal amount of sperm, but in others copulation may not result in emission of semen because refilling has not taken place. If a sluggish, slow-breeding ram yields an ejaculate of subnormal quality or none at all after a sufficiently long rest period, this can probably be considered a sign that the refilling did not occur because of lack of sperm in the testis or of some grave disturbance in the neuro-muscular mechanism of sperm transportation."

A prolonged sexual rest of males of various species results in the first ejaculate's being of low quality. Trials have shown that where the interval of use ranged between six hours and a week, the volume of semen and the sperm concentration were not appreciably affected. Lambert and McKenzie (1940) found that most mature stallions could be used from two to five times on some days without reducing their fertility, but that they should be used much less frequently after a few days of heavy service. Like other animals, stallions differ widely in their semen-producing capacities. The older ones usually show sluggishness and emit a large proportion of immature sperm sooner than younger horses after frequent services.

According to work done by McKenzie *et al.* (1938), the volume of semen from boars was not reduced by services at 48-hour intervals, but a marked drop occurred at 12-hour intervals. They found no correlation between live-weight and volume of ejaculate. In one trial, four boars ejaculated at the average rate of approximately 20 billion sperm daily for several days, and another, still more sexually vigorous, gave 33 billion daily for 14 days. Collection of semen every 72 hours over a nine-month period appeared to give satisfactory production of boar semen, based on research by Dziuk (1958).

#### SERVICE BEHAVIOR

How to induce service by certain males that are impotent, unable, or unwilling to serve can be a major problem. It is one that has received less attention than it deserves, because unless a male is kept to work, his condition amounts to sterility. The factors that may influence sexual behavior are both external and internal. The internal factors include the physiological—especially those connected

with the endocrine system. These, as well as the part that disease and physical defects can play in service behavior, are treated in other chapters.

Lagerlof (1934) reports the slaughter of 161 bulls in one year on account of sterility. Fifty four per cent were discarded because of inability to serve, and 88 per cent because of lack of desire to mate. Unwillingness of bulls to serve seems to occur more frequently under the artificial than the natural breeding program. Says Henderson (1946), "The usual history of these cases is for the change to take place gradually, the bull requiring a little longer reaction time for each service, until finally service is refused. He may show no interest in the teaser cow or may confine himself to occasional attempts to mount which are not completed. The cause of this condition is not always clear, but may be overwork, rough handling of the artificial vagina, or wide variations in the temperature or pressure of the artificial vagina. In preventing its occurrence, it is recommended that conditions be standardized as much as possible. Service should take place in the same location each day, at approximately the same time, and the space between service dates should be kept as even as possible. Service should not be preceded by a heavy feed. Pressure and temperature of the artificial vagina should not vary between services for an individual bull but may vary between bulls, and care should be taken that the penis is not injured during collection."

With this type of behavior there are various ways of inducing bulls to respond. These include changing the teaser cow, substituting bull for cow, leading the cow away from bull, and further frustrating the bull by leading him to and from the cow without the opportunity to serve. In the bull, keenness to serve should not be considered a criterion of fertility. Trials have demonstrated, however, that the restraining of bulls to induce sexual excitement can increase semen volume and the number of motile sperm per ejaculate. Some completely sterile bulls will exhibit normal mating behavior. There is good evidence too that in rams the desire to breed is not a wholly reliable index of fertility or of their spermatogenetic activity, although there is some relationship. McKenzie and Phillips (1937) reported trials which showed that many rams continue to copulate without emission of sperm following frequent matings.

Proper training in early breeding life is important in the work of

semen collection Most bulls, rams, and boars can be taught to mount females out of heat

Crombach *et al* (1956), working with identical twin bulls, obtained twice as many living sperm when the bulls were restrained for five minutes and allowed one false mount before ejaculation as when no frustration was practiced Restraining bulls prior to first ejaculation was accompanied by highly significant increases in semen volume per ejaculate, the sperm cell concentration, and motility of sperm cells, as a result of trials by Branton *et al* (1952)

#### DISEASES

It has been mentioned that injuries and diseases of various kinds can influence service behavior They can also directly affect the quality and quantity of semen even when males are able to serve the artificial vagina or copulate A marked rise in body temperature is often quickly reflected in semen production There can be sperm degeneration due to acute abscesses The severe fever that follows can cause the loss of sperm heads Not only the commonly occurring foot-rot in bulls and rams but also severe abscesses and swellings, trauma (inflamed scrotum), epididymitis, and hypoplasia are examples of ailments that impair the breeding powers All valuable sires should be given clinical examinations at regular intervals

#### BACTERIA

The genital tracts of sterile males and those of low fertility often harbor large numbers of various kinds of bacteria, including staphylococci, micrococci, bacilli, coliform organisms, *Pseudomonas pyocyanea*, and *Brucella abortus* New York and New Jersey, investigators found hundreds of millions of bacteria per cubic centimeter in many samples of bull semen

Knodt and Salisbury (1946) have showed that in normal ejaculates from bulls, optimal density of bacterial growth doubles in three days The artificial breeding organizations now add penicillin or streptomycin or both to bull semen diluter The practice of making such additions is highly recommended as a means of preventing bacterial growth and of killing certain pathogenic organisms that may be present in the semen

## TRANSPORTATION

The transportation of certain males may prove injurious to their breeding powers. On the other hand, there are reports of long-distance shipments exceeding a thousand miles by truck and railroad with no apparent ill effects on ability to serve artificially or naturally and no effect on sperm production. There is belief among managers of cattle breeding associations that refusal of some bulls to mount a few days after arrival at another headquarters is due to the new surroundings and not to shipping injury. It is sound practice to allow the males of most species to rest and to become acquainted with their new environment before putting them into service. Willett's studies (1957) show that no reduced fertility in bulls could be attributed to transportation.

## AGE

Even though working with 41 bulls especially selected for their fertility, Tanabe and Salisbury (1946) found that young bulls between the ages of one to three, inclusive, had the highest breeding efficiency of all of the age groups. The two year old bulls that rated the best had 58.1 per cent of non returns to first service, followed by 50.6 per cent for three year-olds, 49.3 per cent for four year-olds, 42.1 per cent for five year-olds, 42.2 per cent for six year-olds, 49.9 per cent for seven year-olds, 51.6 per cent for eight-year-olds, 48.4 per cent for ten year-olds, and 43.1 per cent for twelve-year-olds. From the ages of three to seven years, inclusive, the cows maintained a fairly uniformly high breeding efficiency. The study was based on 12,621 services to Holstein cows in artificial breeding associations.

The investigation by Bowling *et al* (1940) that involved one large herd bred by natural service revealed over a long period an average conception rate of 1.5 services per pregnancy for yearling bulls, 1.63 for two year-olds, 1.65 for three year-olds, 1.56 for four year-olds, 1.79 for five year-olds, 1.87 for six year-olds, 2.05 for seven-year-olds, 2.1 for ten year-olds, 2.68 for thirteen year-olds, and 2.57 for fifteen year-olds. In both of these studies many bulls of mature age had a higher breeding efficiency than the immature ones.

In a study of the work of 353 bulls, McCullough *et al* (1951) did not find a significant trend in conception rate when the age ranged

from two to thirteen years. This does not necessarily prove that advancing age has no effect on a sire's fertility. By paying attention to wise feeding and management, many of the best-bred sires of the different species can be made available for extra years of useful service.

#### INHERITANCE

There is considerable disagreement concerning the degree to which inheritance may be a factor in the reproductive efficiency of livestock, male and female. The prevailing opinion, however, indicates that both heredity and environment exercise an effect. But in most studies, conception rate, however measured, shows low heritability and repeatability.

#### EXERCISE

A comparison between exercised and nonexercised bulls conducted by Snyder and Ralston (1955) showed that exercise did not significantly affect conception rates.

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# Cattle

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Physiological and other difficulties have prevented a wide application of artificial insemination to some classes of livestock; but with cattle many major problems have been resolved. Various improvements in techniques, however, still remain to be discovered.

Leading breeders of dairy cattle were among the first to become interested in the possibilities of artificial breeding as an instrument for herd improvement. They realized full well not only the work, expense, and danger involved in raising and handling bulls but also the highly speculative aspect of the too common practice of mating an entire herd to an unproved sire for two or three years. The chapter on artificial breeding organizations reveals the substantial progress made to date with dairy cattle. Improved techniques and a keener appreciation of the part that good inheritance plays in herd improvement should bring about future marked expansion, organizationally and privately, in use of both dairy and beef bulls.

Since 1952, frozen semen has become an increasingly important part of the cattle breeding program in many countries of the world. Semen processing, storing, and use are fully treated in subsequent chapters.

A knowledge of the anatomy and physiology of the male and female reproductive organs is essential in the proper practice of insemination. For the facts on this subject see Chapter 2, which deals primarily with the reproductive organs of cattle.

## SEMEN COLLECTION

The three best-known methods of obtaining semen from a bull are: (1) by use of the artificial vagina, (2) by electroejaculation, and (3) by massaging of the accessory genital organs (ampullae of the ductus deferens).

*The Artificial Vagina.* The use of this device has proved a great boon in the artificial program with cattle, because with it a normal ejaculate can be quickly obtained. The outfit usually consists of an outside rubber cylinder 16 inches long and 2½ inches in diameter for mature bulls of normal size, and 10 inches to 14 inches long and 2 inches in diameter for those younger or undersized. This stiff casing is fitted with an inner rubber lining, the ends of which are turned

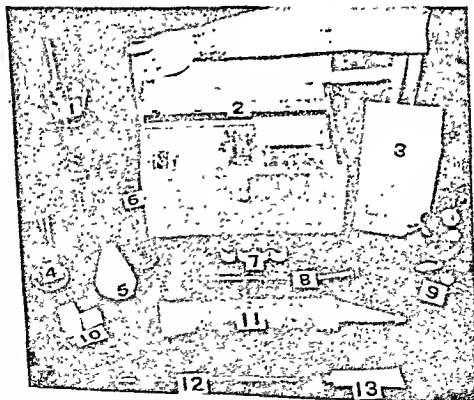


Figure 19. Artificial insemination equipment for cattle. 1. Erlenmeyer flask for filling vials. 2. Inseminator's kit. 3. Semen shipping carton. 4. Pharmaceutical graduate with ounce and metric rings. 5. Pyrex flask. 6. Graduate. 7. Semen dyes. 8. Syringe and inseminating tube. 9. Microscope. 10. Antibiotics. 11. Artificial vagina. 12. Lubricating rod. 13. Lubricant.

back over the cylinder ends and held in place with heavy rubber bands. A funnel-shaped piece of rubber, holding a graduated test tube or vial in its small end, is slipped over one end of the vagina, as shown in Figure 19. The tube is covered with heavy cloth to protect against breakage. Through the hole or special valve, the jacket is filled one-half to two-thirds full with hot water at 130° to 150° F. The optimum is approximately 140° F. The inside temperature of the vagina should be between 105° and 115° F., although the requirement for different bulls sometimes varies. A thin coating of sterile, water-soluble lubricating jelly is then applied to the first four or five inches of the inside anterior part with a sterile glass rod. If the bull fails to ejaculate within a period of six or seven minutes, the vagina should be re-warmed by a change of water. If the collection must be taken any distance it should be tightly wrapped in a blanket. Some technicians, when operating in cold weather, help to protect the sperm against cold shock by using a long type of vagina which contains within it the collection vial. A few individuals prefer an artificial vagina which is distended and pressurized by air instead of water. In this case, the inner liner is usually made of a rather heavy type of rubber with a rougher than usual surface, and it is sometimes used unlubricated. For each ejaculate, use a clean vagina to reduce the possibility of bacterial contamination.

In this method of collection, bull preparation is of special importance. The hairs near the end of the sheath should be kept trimmed to a length of approximately one-half to two inches. The underside of the body must be clean and should be washed with warm water free of soap, and allowed to dry prior to semen collection.

Next in order is the conditioning of the bull, psychologically and sexually, for maximum response as desired by the technician. A quiet cow, bull, or steer is generally satisfactory as the stimulus animal or "teaser." As a means of increasing the quantity and quality of semen, it pays to restrain most bulls just prior to collection. The studies in bull behavior by Almquist and Hale (1958) showed that more than twice as many sperm per ejaculate can often be obtained from a bull if he is restrained, allowed false mounts, or subjected to other teasing. A field study revealed that sperm numbers increased 67 per cent when ejaculates were collected at the rate of two per week instead of one per week. Teasing methods were a factor in in-

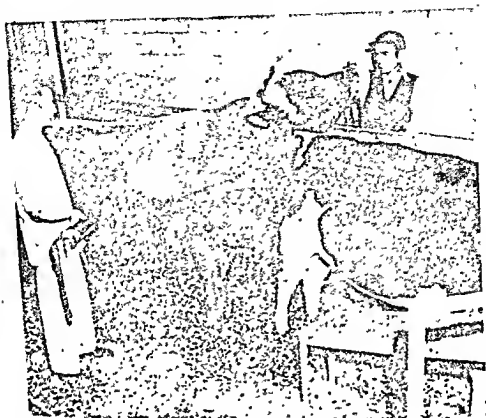


Figure 20. Technicians of a modern artificial breeding organization prepare to collect semen with the artificial vagina.

ducing one bull to ejaculate 77 times in five hours, and another yielded 79 billion sperm within a period of seven hours. The means of stimulating sexual activity included changing the teaser animal, changing the collecting site, circling the teaser around the bull, allowing false mounts, and presenting two teasers side by side.

Prior to making a collection by means of the artificial vagina, the stimulus animal should be placed in a breeding rack (see Figure 20). As the bull starts to mount, the technician holds the artificial vagina alongside the confined animal's thigh with the open end lowered so that he can quickly guide the penis into it by applying his free hand to the sheath. When the bull dismounts, the vagina should be upright at once to protect the semen collection.

**The Electroejaculator.** This is an emergency device with which semen is taken from a crippled or libido-lacking bull by stimulating

the nervous system of his reproductive tract. With the probe in the rectum, the current is started at a low voltage and is increased rhythmically to the point where ejaculation usually occurs. The managers of breeding organizations report that this machine has its definite place but that they seldom obtain a quality of semen equal to that afforded by the artificial vagina. Some of the later units have been reduced in size because of being equipped with resistors. The manufacturers have been trying to devise improvements sufficient to eliminate the objectionable spasmodic reaction of some bulls. The animal can be best controlled in a narrow chute or similarly confined area. Cupps *et al.* (1957) collected semen on alternate weeks for eight weeks by the electroejaculation and the artificial vagina methods from nine Herford bulls sixteen to nineteen months old. The resulting differences in per cent of motile, live, or abnormal sperm were not significant, but the average sperm concentration, total number of sperm per ejaculation, and fructose and citric acid levels in the semen were significantly lower in the semen collected by electroejaculation.

**Massaging the Ampullae.** Another method of collection and one that requires considerable experience and skill is the massaging of the ampullae via the rectum. Before practicing this system, firsthand knowledge should be obtained from someone who is adept at it. Some bulls respond rather poorly to it, and the collections of semen are not likely to be as clean as those made with the artificial vagina. This method therefore is recommended only in the case of valuable bulls that are lame or for some other reason do not mount the cows.

By a few stroking motions, rid the sheath of any residual urine to prevent urine contamination of the semen. Clip the end of the sheath and wash it with water, using a soft brush, partly to induce urination before collection, since urine is toxic to sperm.

The assistant should hold a funnel leading to a test tube directly underneath the prepuce. Several test tubes should be kept on hand so that a clean tube can be substituted if the one being used becomes dirty. The operator must have short fingernails, and it is well for him to wear a long-sleeved rubber glove as arm protection. The anus of the bull and the arm of the operator are well lubricated with mineral oil or vaseline or a mild soap. All feces are first removed from the rectum, and the ampullae are then located by protruding

the hand forward enough so that the fingers fall over the anterior edge of the pelvic bone. Then a backward drawing of the arm and a gentle downward pressure of the hand will let the fingers drop into a triangular space formed by the two ampullae (see Figure 21). The two seminal vesicles join the urethra at the apex of this triangle, and a band of tissue is felt just behind this point (Fig. 21, *c*, *d*, and *e*). The ampullae are massaged by the 'stripping technique'. The second finger of the hand is run between the two ampullae, and the index and third finger are placed on the outer side of the ampullae. A slow, rhythmic motion must be practiced with this massaging method.

**Nonmounting Males** Bulls which refuse to mount for one reason or another can sometimes be trained to ejaculate. The decoy is brought to the bull's pen or other suitable area for his stimulation. After protrusion of the bull's penis and release of some seminal fluid, the person collecting applies an artificial vagina of about eight inches in length to the thrusting penis. McLean (1957) found that a few weeks are usually required to train such bulls to the point of suitable response. The decoys must be changed more often than usual. There are records of satisfactory collections for 18 months or longer, with conception rates almost as high as when normal collecting was practiced.

**The Dummy Cow** Some of the breeding associations have the dummy on hand as part of the standard equipment, but reports indicate that the percentage of bulls that will mount it is smaller than the percentage that will mount a cow in the breeding rack. The degree of success with this apparatus (see Figure 22) depends upon the training given the bull. He should first be allowed to ejaculate, using a teaser cow or male, and he should be taken to the same quarters each time. After he has learned to anticipate service there, the dummy may be substituted for the teaser. Young bulls are more easily trained than the older ones. The dummy must be strongly constructed with the framework preferably of metal and adequate to support the weight of the largest bull. It should bear some slight resemblance to the female, and the top and sides should be well padded and covered with a hide or strong canvas. The vagina can be held in place by a spring like attachment, by straps, or by some one seated under the dummy.



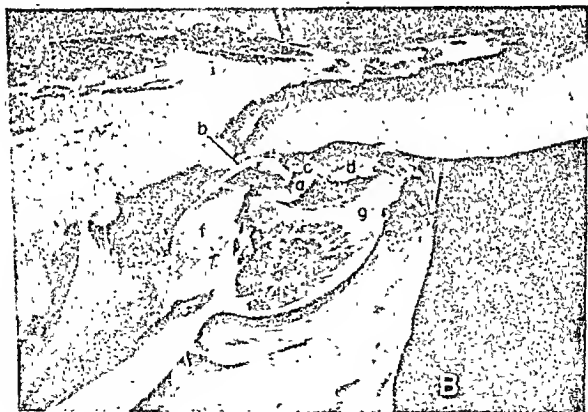
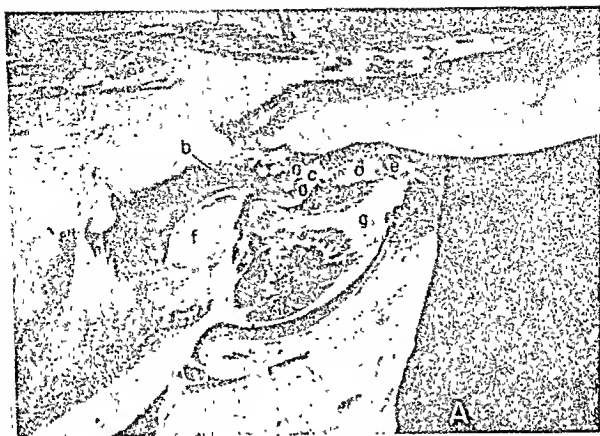


Figure 21. Position of the genital organs of the bull and method of manipulating them: A. Massaging seminal vesicles. B. Massaging the ampullae of the ductus deferens. The organs are: a. Seminal vesicles. b. Ampullae. c. Body of prostate. d. Pelvic urethro. e. Bulbo-urethral (Cowper's) glands. f. Bladder. g. Pelvic bone. (Miller and Evans, 1934.)

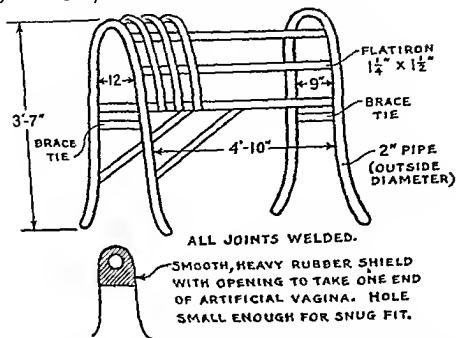


Figure 22 The metal form for the dummy cow is covered with a mattress which is rolled around the ends to give considerable padding of these points. A tanned hide (horse or cow) is stretched tightly over the whole. Hips may be simulated by stuffing padding between the ribs and the mattress or by raising one of the ribs so that it is about two inches out of line with the other ribs. It is well to line the inside of the dummy with burlap or canvas before the hide is attached. The lining keeps the penis of the bull from striking the ribs. (Courtesy University of Wisconsin)

#### CHARACTERISTICS OF BULL SEMEN

Before any bull is accepted by an artificial breeding organization, it must be made certain that he is free of the transmissible diseases. In the case of the younger, unproved bull, it is preferable that he never have been used naturally. Today, many a bull is being leased or bought subject to the passing of various tests, including one for fertility. Both his breeding history and his semen should be carefully examined. A good natural breeding record is hardly sufficient. There is the ever-present possibility that the genital organs may have become quickly infected. Palpation by the veterinarian will reveal whether or not the testes and epididymides are normal.

One or two samples of semen sometimes fail to give a complete picture of a bull's breeding condition. Therefore, many organizations and private breeding establishments prefer to bring him to headquarters where he can be isolated and tested in the various

ways that will indicate the nature of his ejaculates and his service behavior. A high level of sexual activity and sperm-producing ability are desirable conditions governing purchase.

**Volume.** The volume of semen is usually proportional to size and body weight of the animal, although it is something of an individual trait and may even be a family characteristic, as are milk and fat production in dairy cows. Yearling bulls may yield only a few cubic centimeters per ejaculate, whereas older ones often give 6 to 12 c.c. Healthy bulls of both the dairy and beef breeds average 5 to 6 c.c. There seems to be no marked breed difference in mature sires. A normal ejaculate may range from 1 to 15 c.c.

On the basis of moderate usage, some of the difference in volume may be due to the intensity of the sexual preparation—restraint, false mounting, and so on—before ejaculation. Another factor is the varying amount of the more watery portion of the ejaculate known as accessory gland secretions. The main accessory glands, which are the seminal vesicles, vary in size between bulls and are often unsymmetrical in size in the same bull. A little more than half of the ejaculate is from these glands, the function of which is to provide the kind of secretion that will facilitate the ejaculation of the concentrated sperm fraction.

**Color.** Normal bull semen resembles whole milk in color. Its white opaqueness is due almost entirely to the dense mass of sperm present. Grades of semen of lesser concentration are characterized by a more watery appearance, down to almost transparent ejaculates.

Abnormal color can reflect unhealthy genital organs. A yellowish color may indicate the presence of pus or urine, and a reddish or pinkish color, that of blood or degenerating tissue. A watery sample is also unnatural. All such ejaculates signify abnormal semen and should be discarded.

**Motility of Sperm.** When a drop of normal, fresh bull semen is viewed under the microscope, one sees a mass of swirling, progressively moving sperm. As the cooling process begins, motility becomes sluggish and finally ceases. The object of cooling the semen to 35° to 40° F. is to preserve the life of the sperm by conserving their energy and by slowing down bacterial growth. At body or room temperature they become exhausted in a few hours.

**Sperm Concentration.** Ejaculates of heavy concentration are in favor with technicians. The rate of semen dilution is based on the

number of progressively motile sperm rather than the volume of the ejaculate. Normal bull semen ranges in concentration from 300 million to 2 billion sperm per c c, with an average close to 1 billion

#### TESTING SEMEN FOR QUALITY

Wide variation exists in the quality of semen obtained from different bulls. Semen from all extensively used bulls, like those in breeding organizations, should be subjected to tests, some routine and some periodic. It should be understood, however, that there is not yet an infallible criterion of the fertility of the sperm other than actual breeding. Badly needed is a quick, reliable test for semen quality which will predict within very narrow limits the fertility of a given semen sample.

Branton *et al* (1951) published the following statement, based on a series of correlation analyses of semen quality and fertility, using 100 samples: "The following combination of tests and minimum standards are recommended for evaluating bull semen: (a) An initial progressive motility of at least 50 per cent. (b) The concentration as determined by photoelectric colorimeter or hemocytometer should be at least 500 million sperm per c c. (c) The modified methylene blue reduction time, using 300 million sperm per c c, should not exceed nine minutes and preferably not seven minutes." (The methylene blue reduction test has not proved to be as practical and as widely used as the earlier reports indicated; it would be. Details of its techniques can be obtained from the report by Beck and Salisbury, 1943.)

**The Motility Test.** From an examination of 1,000 samples of bull semen, Sferco (1956) noted that sperm motility, as determined visually, was very highly correlated with the per cent of live sperm as revealed by postvital staining. Motility evaluation is based on:

- (1) Per cent motility, which in turn is the visual estimate of the number of actively moving sperm in a semen sample, in terms of a per cent of the total sperm present, in increments of 10 per cent.
- (2) The rate of motility, based on the type and speed of the forward movement of the sperm. The four types of bull sperm motility generally seen are: (a) progressive (b) circular—movement narrowed to a radius approximating length of the sperm, (c) vibratory—side-

to-side motion in static position, associated with aged semen, and (d) reverse—sperm in backward motion.

So important is the test of motility in evaluating bull semen that the method of its estimation is here given in considerable detail, using the techniques of Almquist (1958):

#### I. PREPARATION OF SLIDE FOR ESTIMATION OF SPERM MOTILITY

A. All surfaces which come in contact with semen should be clean and sterile, including glass slides, cover glasses, and glass rods.

B. Warm the slide (on microscope lamp), using care not to get slide too hot. Test by touching it to side of face.

C. Place one drop of warm (98.6° F. or 37° C.) physiological saline (0.9 gm. NaCl in 100 ml. distilled H<sub>2</sub>O) or sodium citrate buffer (2.9 gm. Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>·2H<sub>2</sub>O in 100 c.c. of distilled water) on the prewarmed slide. (If the semen has been diluted to 1:25 or over, no citrate buffer may be needed except with homogenized milk diluent, when the buffer may be helpful.)

D. Invert tube of semen two or three times to mix well. With raw semen, place a small amount of semen in the saline or citrate buffer by barely touching the tip of a glass rod with semen into the buffer. With diluted semen, a larger sample (one drop or a smear the size of a dime) will be needed since no buffer is used.

E. Immediately, place the cork in the tube of semen and put the tube back in the beaker of cold water or into the refrigerator. Do not let the semen in the tube warm up.

F. Gently place a cover glass over the semen sample. With raw semen, first mix the semen and buffer with the cover glass. This glass thins the sample out for better observation and prevents the sample from drying out too rapidly. It also reduces the possibility of smearing the objective of the microscope with semen citrate buffer mixture.

G. Warm the slide to body temperature, 37° C. (98.6° F.). A stage incubator is desirable. The microscope should be kept in a warm room, or it may be necessary to rewarm the slide during examination.

H. If a stage incubator regulated at body temperature is used, it should be permitted to warm up for 15 minutes before a slide of semen is placed in it.

I. For comparison, two semen smears from the same tube of semen should be placed on a slide at the same time.

J. After completing the estimation of sperm motility, place the glass

slides and cover glasses in separate beakers of water for subsequent cleaning

## II USE OF MICROSCOPE FOR MOTILITY ESTIMATION

A With nosepiece adjusted so that shorter objective ( $10\times$ ) is over hole of stage, turn mirror with flat side facing condenser so that it reflects light from lamp through the condenser and through hole in the stage (With a substage lamp, the mirror is not needed) Adjust the mirror until the field in view is evenly and brightly illuminated The condenser should be positioned so that its top surface is approximately one-half inch below the top of stage

B After placing the prepared slide on the stage, grasp the coarse adjustment (large wheel), and watching the objective from the side of the microscope, turn it clockwise until the objective nearly touches the slide

C Look through eyepiece and slowly turn the coarse adjustment counterclockwise to bring the sperm into view

D Manipulate the fine adjustment (small wheel) to bring the sperm into sharp, clear focus

E Examine under the low power ( $10\times$ ) objective by looking at several fields to insure that the semen is evenly distributed and appears the same in all fields Estimate the percentage of motile sperm to the nearest 10 per cent while focusing up and down with the fine adjustment.

F Carefully turn the nosepiece to bring the high power objective ( $43$  or  $44\times$ ) over the field If it appears that the objective will strike the slide, turn the coarse adjustment counterclockwise to permit the objective to come into place

G Focus as above with fine adjustment only Great care is needed because the high power objective is too close to the slide View several fields on each smear

H To help reduce eye strain, keep the eye that is not looking through the microscope open, and alternately use both eyes for viewing

## III COMMON ERRORS IN ESTIMATING MOTILITY

### *Source of Error*

A Human element.

B Flow or drift of sample

### *Method of Reducing*

One person should make all estimations for one purpose *Practice and Core in Insemination*

Use a smaller amount of fluid

- C. Dryness of slide. Have sufficient fluid. Do not allow slide to dry out. Do not use the very edge of the smear. Estimate motility immediately after preparing slide.
- D. Diluter effect. In general, motility is slower in diluted semen containing glycerol, semen diluted in 1:1 egg yolk-citrate, and slightly slower in homogenized milk than in skim milk.
- E. Concentration effect. Use a concentration of about 10 to 40 million sperm per c.c.
- F. Temperature. Have the slide of semen at 37° C. (98.6° F.). Avoid temperature shock at all times.
- G. Sampling. Obtain a typical sample by inverting the tube several times. Use duplicate samples.

#### IV. FEATURES OF AN ADEQUATE MICROSCOPE

- A. Monocular body with 10× Huyghenian eyepiece.
- B. A mechanical stage is preferable but not essential.
- C. Acromatic objectives of the following specifications:

Type	Equivalent focus	Initial magnification	Final magnification with a 10× eyepiece
Low power (dry)	16 mm.	10×	100×
High power (dry)	4 mm.	43 or 44×	430 or 440×

D. Phase-contrast objectives of the same magnifications can be used in place of acromatic objectives. They permit greater ease in observation and greater accuracy in motility estimation.

**Concentration: the Photoelectric Colorimeter.** For present-day breeding organizations the photoelectric colorimeter is the best and most practical means of making sperm counts so that each bull's semen can be so diluted as to insure enough sperm for every insemination. The instrument must be standardized against known sperm concentrations as determined by the hemocytometer method before

using it routinely. If several colorimeters are used in a state or other cooperating area, they should all be so standardized as to obtain similar results and have similar data reported to research workers. They function on the principle that the higher the concentration of sperm the greater will be the opacity or turbidity of the semen. The operator should always follow the instructions of the accompanying manual when setting up and using this equipment. In addition, the following procedures practiced at the Dairy Cattle Breeding Research Center at the Pennsylvania State University should prove helpful.

## I OPERATION OF INSTRUMENT

The following specific instructions are intended only as a supplement to the general instructions contained in the instruction manual furnished with each instrument. Consult instruction manual for the proper setting up and operating procedures.

A Select a location for instrument away from strong light and excessive vibration.

B Permit instrument to "warm up" for a period of *at least 20 minutes* prior to use.

C Do not move or jar instrument while galvanometer switch is in the "Operate" position, or serious damage may result.

D *Always* check the zero position of galvanometer before each series of readings.

E Check the blank reading (100 per cent transmittancy) before *each sample* determination. The blank should consist of 5.0 ml of 2.9 per cent sodium citrate solution (without sulfanilamide) in a clean, dry absorption cell.

F. Use the red filter, 630 m $\mu$ , for all blank and sample readings.

## II DETERMINATION OF SPERM CONCENTRATION

A Pipette 5.0 ml of a 2.9 per cent sodium citrate solution into a *clean, dry* absorption cell (test tube). The citrate solution should be brought to room temperature before use. Use pure crystalline sodium citrate with two molecules of water for preparing the citrate solution. Store the citrate solution in a refrigerator and prepare fresh material at least every two weeks. Immediately after putting in the citrate solution, place a *clean, dry* rubber stopper in each test tube.



B. Add 0.1 ml. of freshly collected semen to the citrate by means of a 0.1 ml. capacity serological pipette. Carefully remove *all traces* of semen from the outside of pipette by wiping it with a clean piece of cheesecloth.

C. Rinse the pipette *at least three times* by drawing up and discharging citrate solution into test tube. Following final rinsing of pipette, blow out the last drop while touching the tip of the pipette to the inside of the test tube.

D. Replace rubber stopper and carefully invert diluted sample *at least five times* to insure complete mixing. Do not shake the tube, as it is important to avoid the formation of air bubbles at the surface of the liquid.

E. Adjust the colorimeter to read 100, using a test tube containing *only* the citrate solution. Wipe the surface of the test tube with a clean, dry piece of cheesecloth before placing it in the colorimeter. The colorimeter must be adjusted to 100 before each reading.

F. Place the test tube containing the semen in the sample holder immediately after mixing, and make the reading. The surface of the sample tube also should be wiped with a piece of cheesecloth.

G. Record the *first stopping point* noted in the swing of the galvanometer. If difficulty is encountered in obtaining a reading, remove the sample, mix the contents again, and replace the tube in the sample holder.

H. "Drifting" or a gradual shifting of the galvanometer may be encountered. However, the instrument is standardized on the basis of the reading procedure described above, and thus it is essential that the first stopping point be used as the colorimeter reading.

I. Refer to Table VI and record the concentration of spermatozoa corresponding to the colorimeter reading.

### III. NOTES

The absorption cells must be carefully cleaned to prevent scratches caused by careless handling and brushing. Scratches on the surface of the glass tend to distort the light passing through the sample and result in inaccurate readings.

Do not remove filter holder from instrument while light is on. Full illumination striking the galvanometer may result in serious damage.

If the filter is broken and a replacement is required, the instrument must be restandardized. Thus, it is highly advisable to obtain a plastic or cloth cover for the instrument at all times. Inspect filter at monthly intervals and carefully clean, but *only* if necessary. Consult instruction manual for the proper method of cleaning the filter.

TABLE VI MAXIMUM DILUTION LEVELS TO USE WITH DIFFERENT AVERAGE CONCENTRATIONS OF SPERMATOGOA

Concentration, sperm/c c (millions)	Dilution level to give in diluted semen (millions of sperm)				Concentration, sperm/c c (millions)	Dilution level to give in diluted semen (millions of sperm)			
	12	10	8	6		12	10	8	6
100	7	9	12	16	1,450	120	144	180	241
150	11	14	18	24	1,500	124	149	187	249
200	16	19	24	32	1,550	128	155	193	257
250	20	24	30	41					
300	24	29	36	49	1,600	132	159	199	266
					1,650	136	164	205	274
350	28	34	43	57	1,700	141	169	211	282
400	32	39	49	66	1,750	145	174	218	291
450	36	44	55	74	1,800	149	179	224	299
500	41	49	61	82					
550	45	54	68	91	1,850	153	184	230	307
					1,900	157	189	236	316
600	49	59	74	99	1,950	161	194	243	324
650	53	64	80	107	2,000	166	199	249	332
700	57	69	86	116	2,050	170	204	255	341
750	61	74	93	124					
800	66	79	99	132	2,100	174	209	261	349
					2,150	178	214	268	357
850	70	84	105	141	2,200	182	219	274	366
900	74	89	112	149	2,250	186	224	280	374
950	78	94	118	157	2,300	191	229	286	382
1,000	82	99	124	166					
1,050	86	104	130	174	2,350	195	234	293	391
					2,400	199	239	299	399
1,100	91	109	136	182	2,450	203	244	305	407
1,150	95	114	143	191	2,500	207	249	311	416
1,200	99	119	149	199	2,550	211	254	318	424
1,250	103	124	155	207					
1,300	107	129	162	216	2,600	216	259	324	432
					2,650	220	264	330	441
1,350	111	134	168	224	2,700	224	269	336	449
1,400	116	139	174	233					

*Other Tests.* Another method of gaining some information relating to semen quality is to store samples of diluted semen in a refrigerator at 35° to 40° F. The samples of good quality should retain their motility for 14 or more days, whereas those of low quality will do so for a few days only:

Although not used widely today by artificial breeding organizations, several other semen quality tests are employed from time to time. These include the methylene blue reduction test (previously mentioned), impedance change frequency, the sperm morphology count of stained preparations, and the dead-alive staining method.

#### HANDLING THE SEMEN

After obtaining a good collection of semen that has been properly protected against cold shock, sunlight, water, or harmful chemical agents, the semen should be put in sterile containers and kept covered as much as possible. The technician should dilute it immediately, especially if it is to be held for longer than two hours. Cool by placing the tubes of diluted semen in a beaker of water at 70° F. and put in a refrigerator set for a temperature range of 35° to 40° F. (2° to 4.5° C.). The rate of cooling will then approximate 5° C. every 20 minutes. Many cattle breeding organizations now practice air cooling of semen by using "walk-in cold rooms," with satisfactory results.

#### SEMEN DILUTION

Dilution expands semen volume so that many cows—sometimes 400 or more—can be inseminated from a single ejaculate. A good diluter also prolongs the life of the sperm. Trials by Swanson and White (1956) indicated that when dilution was done within five minutes after semen collection and when the temperature of the diluter was approximately 80° to 90° F., the motility ratings made initially and for ten days after storage were significantly higher than when the diluter was 60°, 70°, or 100°. Likewise, after freezing, the revival rates were higher from the semen that had the 80° to 90° diluter.

The most popular diluters for bovine semen in recent years have been the egg yolk-citrate diluter and the milk diluter.

**I MILK DILUTER PREPARATION**

(usually made the day before use)

**A Source of Milk**

Obtain milk from a reputable creamery or dairy which can supply homogenized pasteurized milk or pasteurized skim milk (Skim milk fortified with extra milk solids should be avoided, as indicated by the work of Saacke *et al.*, 1955 ) Homogenized milk containing vitamin D or activated ergosterol is all right

**B Heating of Milk****1 Double Boiler Method**

- a Heat the milk in the top portion of a covered Pyrex glass or aluminum double boiler to the safe, minimum temperature of 198° to 203° F (92° to 95° C ) Remove the thermometer, replace the lid and continue to heat for ten minutes
- b Use thermometer to assure that the milk has reached 198° F at the start of the ten minute heating period
- c Cool the heated milk approximately to the same temperature as the semen to be diluted
- d Slowly pour the milk from double boiler, leaving any surface scum on the side of the vessel

**2 Direct Heat Method**

- a In order to use this system it should be possible to regulate the amount of heat A gas burner, such as a Bunsen burner, can be used, or an electric hot plate with a rheostatic type switch Poor results have been reported with the use of an ordinary three speed electric hot plate
- b Heat the milk to a temperature of 203° F (95° C ) and then reduce the heat so that the temperature is maintained at 198° to 203° F Begin the ten minute heating interval when the milk has reached a temperature of 198° F (92° C )
- c A thermometer should be used.
- d After cooling the surface scum usually will cling to the side of the vessel when the boiled milk is transferred to other containers If boiling breaks the scum into small pieces remove them by filtering the milk through a piece of sterile cloth.

**C Comparison of Heating Methods****1 Double Boiler**

- a No danger of overheating on boiling too vigorously

- b. Because the double boiler is covered, there is no danger of excessive loss of water by evaporation if the heating interval happens to exceed ten minutes.
- c. Unless thermometer is used, the results may be poor due to failure to attain the desired temperature.
- 2. Direct Heat (open vessel)
  - a. Faster heating than with double boiler.
  - b. There is danger of losing too much water if heating is too rapid or too long.

#### D. Precautions and Suggestions

- 1. Do not under-heat. Use thermometer and make sure the milk reaches 198° F. (92° C.).
- 2. Do not prolong the heating interval beyond ten minutes when an open vessel is used.
- 3. Do not add unheated milk to the heated milk to be used as a semen diluter, as relatively small amounts exert harmful effects.
- 4. Add penicillin, streptomycin, and coal tar dyes—if used, after the milk has been heated and cooled. The antibiotics aid in milk preservation.
- 5. The penicillin and streptomycin can be dissolved with *heated* milk rather than with sodium citrate buffer solution.
- 6. Milk can be heated prior to use and stored in a refrigerator.

## II. MILK—10 PER CENT GLYCEROL DILUTER PREPARATION

- A. Dilute the semen ejaculate to one-half the final desired volume, either with fresh heated skim milk or homogenized milk containing the antibiotics.
- B. Cool to 40° F. over a period of one and one-half to two hours. (Some operators prefer to cool the semen in bulk instead of in the shipping test tubes.)
- C. Add an equal volume of cooled (40° F.) milk diluter having 20 per cent glycerol by volume. For best sperm livability this is done stepwise, first 20 per cent, second 30 per cent, and finally 50 per cent of the total volume of milk—20 per cent glycerol in three stages at ten-minute intervals. The addition of this milk—20 per cent glycerol fraction can also be made dropwise in a cold room during the cooling process by using a separatory funnel.
- D. The diluted semen now has 10 per cent glycerol by volume. Pour it into test tubes and pack for shipment, since no equilibrating is needed as in freezing semen.

*Note:* For best fertility: (a) Ship and store this diluted semen

in vials and tubes completely filled to protect against agitation and aeration (b) Carry the semen on the road only one day Leave remaining supply in filled tubes in the refrigerator for subsequent days (c) Maintain a uniform storage temperature between 35° and 40° F

### III EGG YOLK CITRATE DILUTER PREPARATION

This diluter, devised by Salisbury *et al* (1941), is prepared as follows

- A. Dissolve 2.9 gm of crystalline sodium citrate dihydrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) in 100 ml of distilled water over glass
- B. Prepare the required volume of egg yolk from freshest eggs after washing them and then soaking them in 70 per cent ethyl alcohol for five minutes and then dry before breaking the shell
- C. Using aseptic precaution remove all egg white from around the yolk, puncture the yolk membrane, and allow the yolk to flow into a sterile glass container
- D. To prepare 50 ml of the diluter, mix together 4 parts by volume of the citrate buffer and 1 part of the egg yolk.

Modifications of these diluters have been used lately, frequently with profitable results According to a 1958 survey of 58 breeding organizations in the United States, 60 per cent were using milk as all or a part of their work of dilution Of these, 16 reported milk glycerol, 13 milk only, and 6 milk-egg yolk-citrate There were 16 that used egg yolk-citrate, 6 the egg yolk-citrate glycerol, and 1 an unnamed diluter Almquist (1954) reported little difference between egg yolk citrate and heated milk (both with antibiotics) as diluters for semen from high fertility bulls However, in the case of semen from bulls of relatively low fertility the semen containing heated milk usually showed a higher fertility rate

Of special significance was the evolving of the milk glycerol diluter Its increasing use was prompted largely by the observations by McLean (1956) and by Flipse and Almquist (1956) McLean found that the adding of glycerol either to egg yolk citrate or to heated homogenized whole milk prolonged the life of bull sperm Flipse and Almquist noted that glycerol at the 5 per cent level as compared to 2.5 per cent, resulted in markedly higher sperm motility in stored semen when either was added to a diluter of egg yolk glycine A little later Almquist (1957) reported the satisfactory maintaining of fertility of bull sperm for at least four days through the use of the milk glycerol diluter With this diluter and twice a

week collections from all serviceable bulls of a stud, the chief advantages are: (1) Selected matings are nearly always possible because of choice of sires. (2) Fewer sires are required. (3) Faster proving of young sires is practiced. (4) Labor and certain other costs are reduced. The system of frozen semen possesses these same advantages plus some others, but it is more expensive to operate.

**Diluter Improvement.** Investigators continue assiduously the task of improving the better-known, more commonly used diluters for bovine semen and of evolving new ones. Foote *et al.* (1958) compared a diluter, CUE, with a 50 per cent egg yolk-citrate control. CUE consisted of 1 part of yolk to 4 parts of a pH-adjusted buffer that contained 1.45 per cent sodium citrate, 0.21 per cent sodium bicarbonate, 0.04 per cent potassium chloride, 0.30 per cent glucose, and 0.937 per cent glycine. The diluter contained sulfanilamide, penicillin, and dihydrostreptomycin. For one-day-old semen the 60- to 90-day non-return rates were practically equal, but for two-day-old semen, the figure for CUE was 75 per cent and for the control 71 per cent.

VanDemark and Sharma (1957) evolved the IVT (Illini Variable Temperature) diluent into which CO<sub>2</sub> is incorporated. The diluted material is then sealed hermetically in ampoules and stored in a dark room at temperatures from 65° to 80° F. Preliminary results indicated a rather good fertility rate for six to seven days. Later VanDemark and Bartlett (1958) reported that when the sodium bicarbonate level was raised, semen stored at 40° F. showed a motility decrease from 71 to 63 per cent, only, in 20 days. With extra glucose, egg yolk, and added catalase in the IVT diluter, one- to seven-day-old semen yielded 10 to 20 per cent higher 60-day non-return rates over those in the earlier large-scale trials. Albright *et al.* (1958) reported milk as a diluter superior to sodium citrate when washed sperm, plasma removed sperm, and whole semen were involved. The combination of milk and seminal plasma surpassed that of sodium citrate and the plasma as a substrate for the maintenance of sperm motility. Roy and Bishop (1954) and Saha and Singh (1958) obtained a significantly longer maintenance of good motility by means of glycine-containing diluters than by egg yolk-citrate, the control.

**Dilution Rate (Insemination Dose).** It is of major importance that semen be diluted in proportion to the number of progressively motile sperm per cubic centimeter of semen. To expand bull power it is

essential that as few sperm as possible be used per insemination, consistent with a satisfactory rate of pregnancy. Much progress has been made toward this goal. Willett and Larson (1952) indicated that sperm numbers take precedence over dilution effect in influencing fertility in dilutions at 1:100 and 1:300. The difference in non-return rates was no longer significant when those rate averages were adjusted to the identical sperm numbers by covariance. Salisbury and Bratton (1948) made dilutions as high as 1:400 without markedly reducing fertility, and they suggested 5 to 10 million sperm per insemination as the minimum. Olds *et al* (1953) reduced sperm numbers from 48 to 4 million per insemination without significantly affecting fertility, but 12 million and more gave a little better results. Using motile sperm concentrations of 5, 10, and 15 million sperm per c.c. (insemination), Bratton *et al* (1954) studied the results with 12,558 first services. The fertility rate was practically the same with the two highest concentrations but fell from 70.9 to 66.7 per cent when motile sperm numbers were reduced from 10 to 5 million per insemination. In most parts of the world the average dosage of 1 c.c. of diluted semen seems to be the standard for cows, with a probable range of 0.5 to 1.5 c.c. In the trial by Olds *et al* dosages of 0.25, 0.5, 1.0 and 2.0 c.c., containing 16 or 24 million sperm, produced pregnancy rates without significant differences. For use in liquid form at least 10 million motile sperm per ml. of diluted semen is recommended. Semen for freezing should have a 30 to 35 per cent heavier concentration.

**Antibiotics** All semen contains varying numbers of bacteria at time of collection, sometimes in the hundreds of thousands. The addition of antibiotics is an effective measure, both to control bacterial growth and to increase the conception rate of some of the bulls of lower fertility. Research has revealed it is sound procedure to use 1,000 units of penicillin and 1,000 units or micrograms of streptomycin per milliliter of diluted semen. Satisfactory types are crystalline potassium penicillin "G" and dihydrostreptomycin sulfate. If only one of these antibiotics is to be used, the streptomycin is preferable, but an effort should be made to use both. It is best to add the antibiotics to the diluter prior to combining it with the semen. Unused portions of streptomycin can be stored in the refrigerator for 14 days without deterioration and the penicillin for 7 days.



**Storing Liquid Semen.** The optimum storage temperature for liquid bull semen is approximately 35° F., but 38° to 40° F. is easier to achieve. The 40° F. point should be considered the upper limit. At such holdings the bacterial growth is kept at a minimum. Sperm survival and duration of motility are not effected by the settling out of seminal plasma or egg yolk during storage, but a diluted sample should always be gently mixed before evaluation and also just prior to use for insemination.

**Coloring Semen for Breed Identification.** The semen of bulls of different breeds cannot be distinguished by any characteristic of the semen itself. Hence in the important work of identification, breeding organizations now add coal tar food colors. This procedure, as reported by Almquist (1946), does not affect the livability and fertility of the sperm. Colors generally in use in the United States as recommended by the National Association of Artificial Breeders are:

Ayrshire—Purple  
Brown Swiss—Brown  
Guernsey—Yellow

Holstein—Green  
Jersey—Red  
Beef Breeds—Orange, White,  
Blue, etc.

#### INSEMINATION OF COW

The syringe, now generally the plastic bulb type instead of glass, is connected with a 16-inch plastic or glass tube having an outside diameter of 6 mm. and an inside diameter of 1 mm. For heifers one with an outside diameter of 5 mm. is preferable. The plastic variety is more popular because it bends without breaking, and the price is so low that it is discarded after one use, thus saving labor.

There are two chief methods for the insemination of cows. They are the rectovaginal, or cervical fixation, method and the speculum method. Since 1940 the former has rapidly replaced the latter.

**The Rectovaginal Method.** The principal steps for the technician are: (1) Wear obstetrical clean rubber or plastic glove and sleeve on right arm. (2) Adjust bulb or syringe to the inseminating tube and draw in 1 ml. of semen. (3) Hold this loaded equipment temporarily in horizontal position in mouth. (4) Lubricate glove and sleeve with soap and water. (5) Carefully insert the hand and arm into the rectum for removal of the feces. (6) Clean the exterior of the vulva with cotton or paper towel, and the inside edges of the lips of

the vulva with clean cotton (7) With left hand insert tube into the vulva as far as possible without touching the side walls (8) Avoid urethra by passing the tube along the top of vagina as far as the cervix (9) With right hand in rectum press down on the rectal wall grasp the cervix and with the aid of the little finger and thumb around its posterior end guide the tube into the os uteri (10) Combine gentle pressure of the tube with manipulation of the cervix until the desired penetration is obtained (11) Deposit the semen in the mid cervix or in the body of the uterus

**The Speculum Method** The other method of insemination sometimes used involves the glass or metal speculum. This equipment is lubricated aseptically and carefully inserted in the vagina (see Figures 23 and 24) After the cervix has been located with the aid of a flashlight the inseminating tube can be inserted into the os uteri or posterior part of the cervical canal at an average of 1 to 3 cm

By the rectovaginal method the skillful technician can implant the semen more deeply in the mid cervix or into the uterus and the necessity of disinfecting the speculum between cows is obviated



Figure 23 The speculum method of inseminating a cow showing position of instrument at time of discharge of semen a Walls of vagina cut away b Speculum c Inseminating tube containing semen placed in cervix d Cervix (W W Geen and L M Winters Minnesota Agr Expt Sta Bul 336 revised)

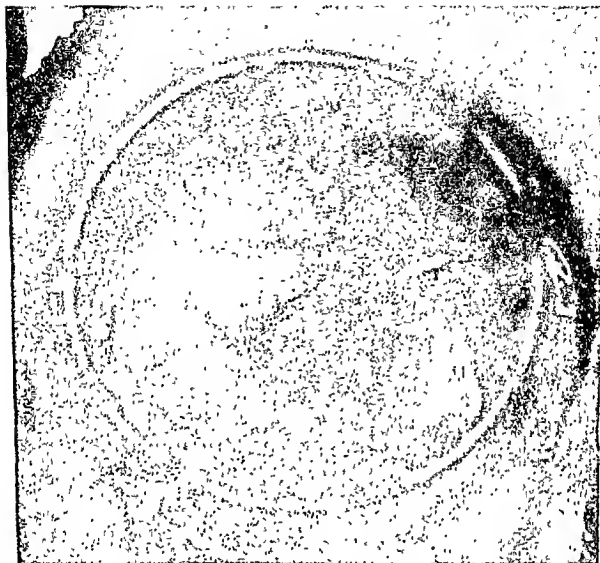


Figure 24. A normal cow cervix, as seen through a speculum. (W. W. Green and L. M. Winters, Minnesota Agr. Expt. Sta. Bul. 336, revised.)

The survey of fertility data by VanDemark (1952) indicated that a 7.7 per cent higher conception rate was achieved by the rectovaginal method as compared with the speculum.

**Point of Deposition.** For first service cows the inseminating tube is usually passed through the cervix and just into the body of the uterus, where one-half to three-fourths of the semen is deposited. Then the tube is withdrawn to the mid-cervix, where the remainder of the semen is left.

For all repeat inseminations, it is deemed advisable to insert the end of the tube only halfway into the cervix and deposit all of the semen there in order to avoid interruption of possible pregnancy, because some cows manifest heat after conception has occurred.

Some technicians formerly practiced placing the semen in the uterine horns—about one half of it in each. This procedure is no longer believed justified because of the discovery by VanDemark and Moeller (1951) that the sperm could reach the ovarian end of the oviduct in a matter of minutes (as low as 2.5) after being placed in the cervix of the cow. The stimulation resulting either from artificial or natural insemination induces the release of oxytocin from the pituitary gland, and this in turn causes uterine motility and the resulting rapid transport of the sperm. Emmens and Blackshaw (1958) studied the comparative effectiveness of the three most common sites of deposition of the semen in the artificial breeding program with cows, based on non returns 60 to 90 days after insemination. The sites, the average fertility results, and the number of first services involved were: (1) Cervix, 64.7 per cent for 6,796 services, (2) Body of Uterus, 65.6 per cent for 6,603 services, and (3) Horns of Uterus, 66.3 per cent for 6,643 services. Stewart and Melrose (1952) gathered data on two sites as follows: (1) Mid-cervix, 64.3 per cent for 4,279 services, and (2) Body of Uterus, 64.6 per cent for 4,554 services. The difference in these various figures was not significant.

The other factors to remember in deciding the site of deposition are the hazard of pregnancy interruption and uterine infection. As early as 1943, Donald reported a study in which 3 to 6 per cent of pregnant cows manifested estrus and that it usually occurred in the first 90 days after conception. This was later confirmed by Donoho and Rickard (1955). Since antibiotics have become a part of practically all semen diluters for the cattle program around the world, numerous technicians, as previously stated, deposit half or more of the semen in the uterine body and the remainder in mid cervix on first insemination. For repeat services the chosen site is the mid-cervix for all of the semen. The work of VanDemark *et al* (1952) indicated pregnancy interruption when semen without antibiotics was placed into the uterus, but intracervical insemination could be practiced without danger. Tanabe *et al* (1955) discovered that bacteria played a major role in pregnancy interruption when semen free of antibiotics was deposited into the uterus, and that many types of organisms were present in the uterus of the cow slaughtered 6 to 15 days thereafter. With antibiotics present, the bacteria were few if any. In pregnancy interruption the mechanical factor was minor. Some day the technicians advocating the different points of

deposition may agree upon a standardized procedure, based on additional data from research specialists.

#### EFFICIENT BREEDING IN DAIRY COWS

A study by Asdell (1957) revealed that faulty management by the cattle owners was the major cause of unsatisfactory conception rates. Part of his data is based on a survey of 19,000 cows in artificial breeding organizations. This study supported the contention that cows first noticed in heat in the morning should be bred on the afternoon of that day, and those first showing heat in the afternoon or evening should be bred the next morning. The inquiry also showed that 21 per cent of the cows were inseminated before the expiration of 60 days after calving, and 37 per cent sooner than 70 days—this despite the fact that much publicity has long been given to the point that the optimum period for conception is between 70 and 90 days after freshening.

Some of the other weaknesses in management include failure to detect heat, especially in winter, the keeping of inadequate breeding records, failure in having regular examinations for pregnancy, and neglecting to call the veterinarian for early treatment of cows afflicted with breeding troubles. The discussion below has a bearing on these and other factors:

*Time of Insemination.* Every day thousands of cows are bred at different stages of heat, some according to plan and others without calculation. The highest rate of conception from single services is reportedly obtained when they are bred in the middle or latter half of the heat period. Theoretically this is correct, but in recent years the conception rate being obtained by those technicians who inseminate the cows on the basis of travel time and without regard to the reported time of onset of heat has been surprisingly good. In fact the resulting non-returns frequently approximate those of the men who adhere to the orthodox rule. The factors responsible could include the several hours of latitude available in relation to ovulation and insemination, greater accuracy in the reporting of onset of heat by the cattle owners, and decided improvements in the techniques of processing, preserving, and using the semen.

Ovulation, the discharge of the egg from the ovary, occurs approximately 12 hours after the end of heat. In a breeding experiment



by the Nebraska Experiment Station (Trimberger and Davis, 1943) 295 dairy cows and heifers were inseminated at various stages of heat to determine the effect of time of service upon conception. The results, expressed as percentages of conception from a single insemination, were as follows: start of heat, 44, middle of heat, 82.5, end of heat, 75, 6 hours after heat, 62.5, 12 hours after heat, 32, 18 hours after heat, 28, 24 hours after heat, 12, 36 hours after heat, 8, 48 hours after heat, none.

By determining ovulation through rectal palpation of the ovaries every two hours, Trimberger (1948) was able to gauge the effect of inseminations at different intervals before and after ovulation. The results were

<i>Insemination time interval</i>	<i>Per cent conception rate</i>
6 to 24 hours before ovulation	79
6 hours or less before ovulation	57
More than 24 hours before ovulation	53
12 hours or less after ovulation	32

The best time for insemination must be based on the time when heat begins or is first noticed because the occurrence of ovulation cannot be exactly predicted.

The approximate range in the heat period of cows is from 3 to 28 hours and averages about 18 hours. It varies widely with different cows, but each cow will usually have about the same length of period every year. Most cows, however, have somewhat longer heat periods during the natural breeding season, when the days are long and the environment is favorable, than in the other seasons of the year.

Two inseminations of cows in one heat period, 10 to 24 hours apart, are sometimes advisable, but because of the extra time and cost involved, it is seldom practicable under the conditions existing in an artificial breeding organization. Cows showing abnormal discharge should not be bred. A bloody discharge on the rear of the animal shows that ovulation has occurred. It bears no relation to conception. Cows do not menstruate. Autrup and Rasbech (1953) reported that when 293 cows were inseminated in the hemorrhagic period about 48 hours after the end of heat, 87 (29.69 per cent) conceived. The

authors explained that delayed ovulation may have occurred or the ovum survival might have been longer than commonly supposed

*Interval after Calving* Mass data from breeding organizations are affording confirming information on the problem of the length of time between calving and the first service thereafter. The figures (Table VII) from Edwards (1951) are from breeding records in England and Wales

TABLE VII CONCEPTION RATES IN RELATION TO INTERVAL BETWEEN CALVING AND FIRST SERVICE

<i>Interval between calving and first service (days)</i>	<i>Number of cows</i>	<i>Per cent of cows conceiving from first service</i>
0-30	290	48.3
60	2,012	62.5
-90	4,431	73.1
-120	3,068	71.5
-150	1,265	73.4
-180	619	69.7

*Breeding in Irregular Heat Periods* A good many cows return to estrus at intervals that vary widely from the expected 21-day period. Some breeders believe the chance of conception in such instances is so slight that the effort is not worthwhile. Available data show that this is not the case. Moeller and VanDemark (1951) gathered information on 4,885 cows in the Southern Illinois Breeding Association, revealing the time interval between two consecutive artificial inseminations for 3,173 cows between first and second, and 1,712 cows between second and third. Four per cent of the returns for service were between 2 and 17 days, 8 per cent between 28 and 35 days, 21 between 36 and 50 days, and 11 per cent between 51 and 72 days after the first insemination. Within a large population the rebreeding of apparently normal cows, regardless of time lapse after previous insemination, is likely to result in an increase of approximately 20 per cent in cows settled in any one estrus.

*Health of Cow* Both a fertile cow and fertile bull are necessary to conception. When an outbreak of sterility occurs in a herd, the owner is usually inclined to blame the sire. Any disease of the reproductive organs of the cow, as well as of those of the bull, increases the number of inseminations required, or may spell sterility alto-



gether There are still plenty of problems of herd health to challenge the cattle owner, veterinarian, breeding technician, and investigator Tanabe and Casida (1949) found a 65.1 per cent embryonic death rate within the first 34 days after pregnancy in their study of 104 repeatedly bred but apparently normal cows Tanabe and Almquist (1953) discovered an embryonic death rate of 54.1 per cent between 3 and 30 days in an experiment with 200 repeat breeding heifers

Crossbreeding repeat breeders, that is, using a bull of another breed on a hard breeding cow, does not increase the conception rate In a study of 6,509 cows, Olds and Seath (1950) noted that their breeding efficiency could not be well predicted on the basis of previous conception rate Approximately only one of ten herds that were hard breeding one year continued to be a trouble maker the next Dunbar and Henderson (1950) have reported the heritability of fertility in dairy cattle to be near zero

Barrett *et al* (1948) discovered that the conception rate based on 60 to 90 day non-returns to service was approximately 5.5 per cent higher than the rate based on actual pregnancy examinations According to carefully gathered reports, about 10 per cent of the cows of nearly all herds give evidence of breeding trouble at some given time Routine pregnancy examinations and veterinary treatment for hard breeding cows pay dividends

**Nutrition.** Nutritional deficiencies are sometimes considered the cause of low conception An answer to the question of the possible relationship between nutrition and fertility in cattle is being attempted through a long-range regional project in the United States The different levels of energy intake, the variation in quality and amount of protein, and the role of trace minerals on the fertility of male and female are examples of factors being evaluated The report of Bratton (1957) on a study of the results to date indicates no grounds for the earlier opinion that fertility may be badly affected, either by an unusually low or by an unusually high intake of feed nutrients in the period of early growth of the animals A similar conclusion is also cited with reference to dairy cow rations low in trace minerals and to young bull rations with varying qualities of protein The trials, however, have clearly shown that when the nutrient consumption is so low as to stunt growth and normal development, the initiation of the reproductive function is retarded

**Age of Cow.** Virgin heifers require more services, artificial or natural, on the average, than older cows Yet in some herds the fertil

ity of the heifers is satisfactory. Most successful breeders avoid a high condition of flesh in all heifers of breeding age. The highest rate of conception in dairy cows usually occurs from the third to the sixth pregnancy. Studies with heifer cows show that fertility is generally lowest at four years and highest at nine years of age.

**Age of Semen** In the interest of breeding efficiency, the age of liquid semen should be carefully noted before use. It can be used profitably up to four or five days of age if processed according to the methods previously outlined. The maximum age at which bovine semen properly frozen and stored can be satisfactorily utilized has not been finally reported.

**Mixed Semen** Trials by Campbell and Jaffe (1958) revealed that the mixing of semen from several bulls did not improve semen quality—contrary to a belief in some areas of the world. In fact 83 of 104 mixed samples had a motility score less than that for the best individual constituent sample.

#### PRACTICAL RECOMMENDATIONS

These steps suggested by G. W. Trimberger of Cornell University are the ones that owners of high breeding efficiency herds are trying to follow to the best of their ability.

1. Keep records of freshening dates, heat dates, service dates and other observations. These provide the information necessary for a high breeding efficiency. They help to detect irregularities and are of aid to the veterinarian in diagnosing trouble.

2. Turn open cows out *twice daily* and *observe carefully* for heat periods, especially 18 to 22 days after a previous period.

3. Cows should not be bred until at least 60 days after freshening. Earlier service results in a low conception rate and poor reproductive health of the cow at the next calving. One or two "free" heat periods without service during the first 60 days after freshening is very helpful to get the cow's reproductive organs in good condition for conception.

4. If a cow has had trouble in calving, has retained the placenta or showed an abnormal discharge after calving, have her examined and treated by a veterinarian until her genital organs are normal before breeding her again. A delay in breeding is usually required for such cows.

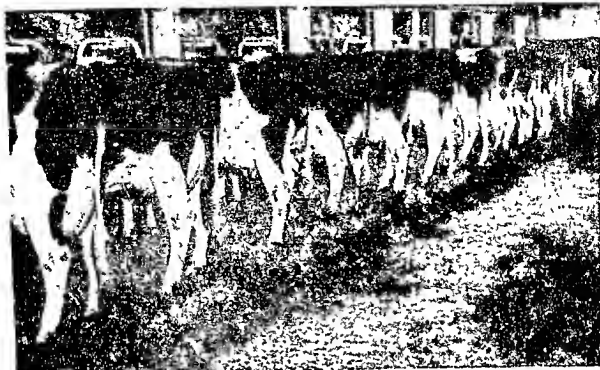


Figure 26. Uniformly superior daughters of a noted proved Holstein sire Oakcrest Raburke Dean. (Courtesy American Breeders Service.)

5. If a cow does not show heat so that she can be bred at the time planned, have her examined promptly by a veterinarian. Any cow which has been fresh 80 days without indicating heat should be reported to the veterinarian so that he can induce estrus. Early diagnosis and treatment of breeding troubles are extremely important.

6. Avoid service during the first six hours of heat. Cows *first observed* in heat in the morning must be bred the same day for good results. If bred the next day, results may drop as low as 30 per cent for conception from the first service. Cows *first observed* in heat in the afternoon and which definitely were not in heat in the morning should be bred the next forenoon. For artificial breeding organizations such females are reported as "P.M." cows. The following time table should be followed for best results in breeding dairy cows artificially:

<i>Heat first observed</i>	<i>When to breed</i>	<i>Too late</i>
In the morning	The same day	The next day
Afternoon or evening	Forenoon next day	After middle of afternoon the next day

7 A supplementary rule to 6 is that *dairy females must be bred not later than 10 hours after the end of heat*. The length of heat ranges from 3 to 28 hours and averages 18 hours. However, 90 per cent of the females are in heat from 10 to 24 hours. Proper timing is important because the sperm cells live only from 24 to 30 hours in the cow. *The egg is usually released between 7 to 11 hours after the end of estrus, and it remains fertile only a few hours*.

8 If a cow does not conceive from the first three services, do not breed her again until she has been examined by a veterinarian. A sexual rest period without service often aids conception. Breed again when her genital organs are in good condition.

9 Breed females to a fertile sire when they are normal, healthy, and ready to be bred. Examination by a veterinarian may be required to determine both of these conditions. Both sires and females have different levels of fertility. *As fertility in one sex decreases, the fertility in the opposite sex becomes of greater importance*. As females decrease in fertility, the most fertile bull available, whether in natural or artificial breeding, should be used in the effort to obtain the desired conception.

10 Since heifers are often more difficult to get with calf than older cows, it is recommended that they be given large amounts of high quality roughage and otherwise be permitted a health promoting environment.

11 Dry cows off 60 days before calving and have them in good physical condition at time of calving.

12 Maintain sound reproductive health in cows by providing the following: mineral box with needed minerals, plenty of exercise, proper housing, feed and comfort, *care of feet*, protection from various infections that are bound to occur on every farm, isolation for cows aborting, *no tonics unless recommended by a veterinarian*, and breeding only to bulls of high fertility.

13 As a guide in following the above recommendations, it should be recognized that a conception rate of 60 to 70 per cent from the first service or 80 to 90 per cent pregnancies from three services is a very desirable breeding efficiency. Normally it is expected that from 5 to 10 per cent of the females are sterile. It is therefore reasonable to expect that one cow will be sold as sterile each year for every ten to twenty females in the herd.

## • HEAT PERIOD DETECTION

Certain owners and herd managers are much keener at this than are some others. The cow with the "quiet" heat is the trouble-maker. The chief signs of heat in cows and heifers are these:

1. Standing still while the other cattle ride them—usually a sure indication.
2. Riding other cattle—although cows not in heat will frequently ride those that are in heat and the cow with "quiet" heat will do little or no riding.
3. Bellowing and nervousness and attempting to ride the animal in the adjoining stall. Reaching toward other cows or herdsman.
4. Clear, watery secretions from the vagina.
5. Some hair on tailhead and rump usually ruffled and rubbed off.
6. Decrease in appetite, and irregular milk production, usually a decrease.

## A REASONABLE CONCEPTION RATE

What rate of conception should be expected in well managed dairy herds? Perhaps as good an answer as any is afforded by a breeding analysis of the 500-cow herd at the Clemson Agricultural College in South Carolina (Hernan, 1959). The rate of conception is based on pregnancy diagnosis after artificial insemination. The results covered the two-year period from 1955 to 1957 and were as follows:

1. Of the 660 cows inseminated, 591 (90 per cent) finally became pregnant.
2. Fifty-six per cent of all cows became pregnant on first service.
3. Following second service, 74 per cent of all cows were pregnant.
4. Following third service, 81 per cent of all cows were pregnant.
5. The average number of services for observed pregnancies was 2.0. A few cows were given fourth and fifth services, and still fewer were inseminated up to nine times.
6. Contrary to some evidence in other surveys, the rate for the 316 heifers excelled slightly that of the cows, being 1.9 services per pregnancy. The total per cent becoming pregnant was 97. Two per cent of the others were adjudged infertile.

7 Two per cent of the cows that failed to conceive were sold as sterile. The others were disposed of for various reasons.

The above results are good for so large a herd. Most owners are well pleased when 85 to 90 per cent of their cows are in calf after three services. Almost invariably some cows require a fourth or fifth service. It is not usually considered economical to service after the fifth. Many owners resort to examination by a veterinarian when the results are negative after third service.

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# Buffaloes

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P BHATTACHARYA

The domestic buffalo native to the East (*Bubalus bubalis*, L., or *Bos bubalis*) and the domestic cattle of Europe and America (*Bos indicus* and *Bos taurus*) have similar utility to mankind, yet the former has received much less attention, and as a consequence comparatively little information on buffaloes is available in the literature. Publication on artificial insemination in buffaloes is indeed extremely meager. As so little is known about the buffalo outside its habitat, some information on its geographical distribution, importance in agricultural economy, potentiality of development, and so on, is given here before discussing artificial insemination and related matters in this species, for these have some bearing on the organizational aspects of artificial breeding.

There are wild and domesticated buffaloes and the domesticated ones may be classified in two main categories: the swamp buffalo and the river buffalo. Though these two types belong to the same species, their habits are very different, and they cannot be crossed with advantage. There is no authentic information about the buffalo being *successfully* bred with other animals of the same genus, and many believe that this cannot be done. This peculiarity of the buffalo precludes the possibility of introduction of desirable genetic characters in this species from other members of the same genus, such as bison, yak, gayal, dairy or beef bull.

In contrast with cattle, which are distributed all over the globe,

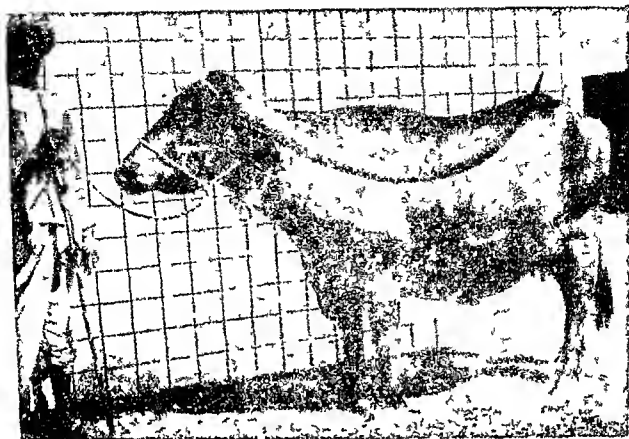


Figure 27 A long horned female buffalo of the Pandharpuri breed, India

the domestic buffalo is to be found only over certain regions of the earth in the Far East, Middle East, and in a few countries of Southern Europe

The world population of domestic buffalo is 70 million, as compared with 541 million cattle (FAO, 1947), but they contribute a great deal to the agricultural economy of the areas where they abound. Like cattle, buffaloes are useful for milk, work, and meat. The relative importance attached to these qualities, however, differs from place to place.

In India, there are 48 million domestic buffaloes of the river type—the largest concentration of buffalo for any one country. These are greatly valued as dairy and work animals. Of the total milk production in the country, 55.9 per cent comes from the buffalo, though they constitute only 30 per cent of the milch cattle. The Indian buffalo, on an average, produces 1,100 lb of milk in a lactation, as compared with 413 lb for the Indian cow. The Murrah breed of buffalo produces 3,000 to 5,000 lb of milk during a lactation period of nine to ten months, and individuals yielding 10,000 lb of milk or more are not uncommon (Phillips, 1949). The fat content of

buffalo milk is much higher than that of cow's milk. It contains 6 to 7 per cent fat, as compared with 4 to 5 per cent for the cow.

In Malaya, Singapore, the Philippines, and several other countries in the Far East, swamp buffaloes exist in good numbers. They are poor milkers but are valuable animals for haulage and cultivation. Their large feet, slow movement, and heavy draft capacity make them particularly suited for paddy cultivation in swampy, water-logged rice fields. The quality of buffalo meat is not as good as that of beef.

In the Middle East, where only the river buffalo is available, the animals are valued for all the three purposes, milk, work, and meat.

In the countries of Southern Europe only river type buffaloes are available, they are mostly used for milk, and only in a limited way for work or for coarse meat.

With good pasture grass and with adequate concentrates, cattle are more efficient and economic producers of milk and meat, but the buffalo has some characteristics that make it more suitable and economical under many conditions of underdeveloped agricultural economy. The buffalo is not discriminating in foraging and can thrive with a minimum of attention. It can produce even when kept on coarse pasture and other roughages, such as sedge, reed, bush, and forest growth of the tropics. The stock owners in these regions maintain buffaloes, not from ignorance of the possibilities of other cattle, but because they find that in the prevailing agricultural situation no other domestic animal will thrive like the buffalo and be so economical. There is no doubt that for a long time to come the domestic buffalo will continue to have a very important position in the agrarian economy of these areas, and, therefore, every attempt to improve the quality of these animals should be made so that they will be more valuable. There is a great deal of genetic variability in the existing stock of domestic buffaloes and this offers a wide scope for selection and improvement. Application of artificial insemination can play a significant role in facilitating and accelerating the process of development.

In many ways, the physiology of reproduction of the buffalo is similar to that of the bull, yet in several aspects of the reproductive phenomenon the two species differ. To achieve best results, the knowledge of artificial insemination gathered from the bull cannot, therefore, be applied in the buffalo without some modifications.

In this chapter an attempt has been made to bring out, from the existing knowledge, the points of difference between the two species, rather than deal with different aspects of artificial insemination in detail.

#### ANATOMY OF THE REPRODUCTIVE ORGANS

**Male.** The external genitalia of the male buffalo are similar to those of the bull. In the buffalo of the river type, the penis hangs in a six- to twelve-inch pendulous sheath formed by a triangular fold of skin extending from the umbilicus backwards, in much the same way as in Zebu cattle. The swamp buffalo has the penis contained in a sheath which adheres close to the body except at the umbilical end, where it hangs free by an inch or so, as in European cattle. In the buffalo, there is no tuft of hair at the preputial opening.

The scrotum in the swamp buffalo is small, being only about four inches when fully extended and has no constriction near the attachment to the abdominal wall. In the river buffalo, the scrotum is larger, with a distinct neck, but even so it is much smaller in size than the scrotum of a bull of similar size.

According to MacGregor (1941), the testicles descend in the scrotum when the buffalo calf is about six months old, but in the Murrah buffalo the writer has observed the presence of testicles in the scrotum at birth. The testicles hang in the scrotum with the long axes perpendicular to the body when relaxed, but when drawn up close to the body by the cremaster muscles, the long axes assume the anteroposterior position. The fully developed testicle of a mature buffalo bull is about half the size of that of a mature bull of European breed. On histological examination the lumen of the seminiferous tubules in the buffalo has been found to be even smaller than that in the ram or the goat (Mukherjee *et al.*, 1957).

The seminal vesicles are also relatively smaller in size in the buffalo than in the bull.

**Female.** The female genitalia of swamp and river buffaloes are similar and resemble those of the dairy or beef cow (MacGregor, 1941). Polding and Lall (1945) made comparative studies on the genitalia of Indian female buffaloes and Zebu cows and observed that the uterus of the buffalo is larger and more turgid than that of the cow. The ovaries of the cows are mottled and freely mobile in a

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loose attachment, but the buffalo ovaries are more tightly secured. The Fallopian tubes of the buffalo are more deeply involved in the broad ligament than those of the cow and are also coarser in appearance.

The uterus of the Zebu is cream colored and faintly tinged with pink in the young animal and yellowish like old ivory in older animals. The buffalo uterus is whiter and stippled with superficial veins not obvious in the cow.

The corpus luteum in the buffalo has a pinkish grey color veined with red, which fades during the regressing phase. At no time during the cycle does the luteal tissue usually attain a yellowish hue. As the corpus luteum regresses the red veining disappears, and the whole tissue turns white and sinks into the stroma of the ovary.

#### MALE BEHAVIOR

It is generally believed that even with good nutrition and management the buffalo attains sexual maturity later than the bull. The buffalo bull usually starts mounting the female at two years of age but fails to serve successfully because of small size. The average age at first service for buffalo bulls in Egypt is 3.7 years (El Itriby and Asker, 1957). In India the bull is put to service at about the same age. Spermatogenesis, however, begins much earlier in this species, and meiotic division of the spermatogonial cells lining the seminiferous tubules has been observed in the Indian buffalo at the age of one year (Dutt and Bhattacharya, 1952). It is likely that the development of spermatozoa begins even earlier.

The buffalo bull is more easily trained to serve the artificial vagina than the bull, any will serve at the first attempt. The buffalo bull is also less discriminating with respect to the teaser and is more likely to mount an anestrous female or a male placed in the service crate. Prabhu (1956) noticed that the reaction time, the period between the approach of the bull to the animal in the crate and ejaculation in the artificial vagina, was shorter when a male rather than a buffalo cow was used as a decoy. There was no correlation between reaction time and the semen quality. The stage of estrus of the female buffalo did not influence the reaction time (Prabhu and Bhattacharya, 1951, Prabhu, 1956). There is, however, a breed difference,



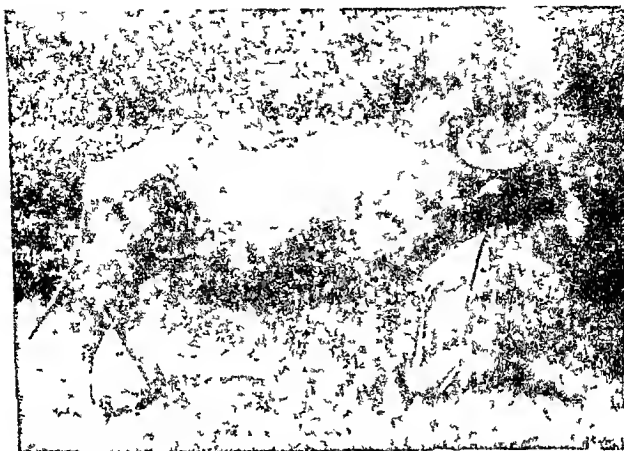


Figure 28 This water buffalo cow of Egypt is a good typical specimen showing considerable dairy character. At the time this picture was taken she was near the end of her lactation period but she has one yearly record of 8 000 lb of milk with an overage butterfat content of 7.5 per cent. This is an extraordinary record but through a well organized program of selection and artificial breeding the germ plasma of such cows can be multiplied to great advantage.

and bulls of some breeds of buffaloes are relatively slow in serving. The thrust given by the buffalo bull at service is much less forceful than that of the bull.

#### SEMEN COLLECTION

**The Artificial Vagina.** The buffalo bull readily serves the artificial vagina, and this is the simplest and best method of collecting semen from this species. Either the long (20 to 22 inch) or the short (14 to 16 inch) vagina can be used. The short vagina is, however, preferable as it is easier to handle and results in less wastage of semen. The method of preparation of the vagina and the technique of collection are the same for the bull and the buffalo.

**The Massage Technique.** Collection of semen from the buffalo by the massage technique is possible, but it is comparatively more

difficult. It appears that the buffalo is less responsive and requires a longer period of training than the bull to donate semen by this method. Failures are not uncommon. Collections have been poorer in volume and sperm concentration and higher in pH than ejaculate collected in the artificial vagina (Gajjan Singh *et al*, 1959).

#### SEMEN CHARACTERISTICS

Semen of healthy buffalo bulls, like that of the bull, is opaque, milky white, or yellowish milky in color, and its consistency may be either thick or thin depending on the concentration of spermatozoa (Shukla and Bhattacharya, 1949, Veeramani Ayyar, 1944).

The spermatozoa of the buffalo are more rectangular than those of the bull, and the pale, stained portion of the head is slightly narrower (MacGregor, 1941). The head, middle piece, and the tail of the buffalo spermatozoon measure on an average  $80 \times 50 \mu$ ,  $125 \mu$ , and  $546 \mu$ , respectively, as compared with  $100 \times 56 \mu$ ,  $139 \mu$ , and  $603 \mu$ , for the bull spermatozoon (Guha *et al*, 1959).

**Volume** The quantity does not usually exceed 50 ml in Indian buffalo bulls, though a volume up to 82 ml was obtained from a Murrah buffalo at the Indian Veterinary Research Institute (IVRI), Izatnagar. The average quantity of ejaculate from the Murrah buffalo is, however, only about 30 ml. In Egyptian buffaloes the volume has been reported to vary from 27 to 40 ml, with a mean of 33.1 ml (Hafez and Darwish, 1956). Madatov (1956) has found an average volume of 3.66 ml for buffaloes in the U.S.S.R. Various workers have reported that in bulls of dairy breeds the average volume of semen is 40 ml. As with the bull there is considerable difference in the semen volume among individuals at different times. Two collections made in rapid succession from the buffalo bull may not show any significant difference in volume (Prabhu and Bhattacharya, 1951), but if four collections are made in rapid succession, the volume significantly drops in the third and the fourth collection (Prabhu and Sharma, 1953).

**Sperm Concentration** The sperm concentration in the buffalo is relatively lower than in the bull (Table VIII). The concentration of spermatozoa in the Murrah buffalo has been reported to vary between 631 and 1,034 million per ml (Shukla and Bhattacharya, 1949, Kushwaha *et al*, 1955), whereas in two of the developed breeds of Indian bulls kept under similar conditions, the average

TABLE VIII. COMPARISON BETWEEN BUFFALO AND BULL SEMEN

Attributes	Buffalo	Bull ( <i>Bos taurus</i> )	Statistical significance
Initial motility	2.5 $\pm$ 0.10	3.4 $\pm$ 0.10	*
Sperm density per ml. $\times 10^6$	1,264.5 $\pm$ 68.4	1,456.3 $\pm$ 62.3	†
Living sperm per ml. $\times 10^6$	831.7 $\pm$ 66.2	1,061.4 $\pm$ 48.4	†
Percentage of living sperm	68.1 $\pm$ 1.0	73.3 $\pm$ 1.4	*
Percentage of total abnormal sperm	17.2 $\pm$ 1.2	15.5 $\pm$ 1.3	
Percentage of living abnormal sperm	8.2 $\pm$ 1.0	7.2 $\pm$ 1.1	

Source: Roy *et al.*, 1958.

\* Significant at 1% level.

† Significant at 5% level.

concentration of spermatozoa was close to 1,500 million per ml. (Shukla and Bhattacharya, 1949). In another study a concentration of 1,264.5 and 1,456.8 million sperm per ml. has been reported for the buffalo and dairy bull, respectively (Roy *et al.*, 1958—Table I). Madatov (1956) found an average of 980 million spermatozoa per ml. of semen in the buffalo in Russia.

**Motility of Sperm.** Based on the examination of a drop of freshly collected semen under the microscope at the room temperature and visual gradations, the initial motility of semen of Indian buffalo bulls has been found to be lower than that of Zebu semen. Using a score extending from 0 to 5, where 0 indicates no motility and 5 the highest, Roy *et al.* (1958) reported an average motility of between 2 and 3 for buffalo semen, compared with 3 and 4 for the Zebu. As the rating of motility by this method is based on the nature and movement of the waves, which are greatly dependent on the concentration of spermatozoa, the lower scoring of the buffalo semen may perhaps be attributable to the lesser number of spermatozoa in the semen samples of the buffalo.

**Hydrogen Ion Concentration.** Very little work seems to have been done to investigate the hydrogen ion concentration of buffalo semen. In one investigation the average pH of Indian buffalo and Zebu

bull semen have been found to be 6.7 and 6.3, respectively (Kushwaha *et al*, 1955). Prabhu and Sharma (1953) observed that when a number of semen collections were made in rapid succession from the buffalo bull, the pH shifted from the acid to the alkaline range.

Gajjan Singh *et al* (1959) have reported a pH of 6.75 for semen collected from the buffalo by the massage technique. A pH of 6.4 for ejaculate obtained in the artificial vagina and a pH of 7.85 were reported by Emmens and Blackshaw (1956), for bull semen collected by the former technique.

**Abnormal Sperm.** Regarding the percentage of abnormal spermatozoa in buffalo semen, the findings of different workers vary. The difference reported may, in part, be due to the difference in the techniques of collection employed by them. Breed differences and the quality of the bulls used may be other factors involved. Kushwaha *et al* (1955) observed that in Indian buffaloes the percentage of abnormality varied from 2.7 to 14.4, with an average of 6.6. Prabhu and Bhattacharya (1951) reported a low percentage of abnormality of 4 to 5, whereas Roy *et al* (1958) found the abnormality as high as 17.2 per cent (Table VIII). In the Egyptian buffalo, Hafez and Darwish (1956) have reported an average of 21.1 per cent of abnormal spermatozoa, with a range of 15 to 32 per cent. Madatov (1956) found the average percentage as low as 3 in buffaloes in Russia, with a range of 1 to 11.5 per cent.

The types of abnormalities commonly encountered in the buffalo spermatozoa are similar to those observed in those of the bull and may involve the head, middle piece, or the tail region. What significance each type of abnormality has or how each type of abnormality affects the fertilizing ability of the spermatozoa is not known, but it is generally accepted that a high percentage of abnormality denotes poor quality of the semen.

**Live and Dead Sperm.** Several investigators have advocated appraisal of the quality of semen samples by estimating the proportion of live and dead spermatozoa contained therein with the use of vital dyes. Lasley *et al* (1942) developed a differentiation technique for bull semen with opal blue. Rao (1958) observed that a large number of stains could be used for differential staining of live and dead spermatozoa in the semen samples of the buffalo and the bull. The use of 5 per cent solution of acid green or china blue or congo rubin or cotton blue or indulin, or 3 per cent solution of bleu de



Figure 29. Normal spermatozoon of the buffalo (*Bos bubalis*) and the bull (*Bos taurus*). Note the difference in size, particularly the longer head and longer tail of the bull spermatozoon (right).  $\times 530$ .

Lyon, or 2 per cent solution of congo red, or 10 per cent solution of light green, or 7 per cent solution of methyl blue, or saturated solution of thionine gave comparable results. Except for congo red and congo rubin, which are used alone, and bleu de Lyon and thionine, the rest of the stains are used along with 1 per cent eosin B solution. With the solutions of bleu de Lyon and thionine, eosin solutions of 0.8 and 0.6 per cent, respectively, are to be used. Reagents like M/8 phosphate buffer, or 3 per cent sodium citrate (dihydrate) solution, or 0.9 per cent sodium chloride solution may be used as satisfactory solvents for the dyes.

Using the method advocated by Lasley *et al.* (1942) and adjusting the buffer to pH 6.6, Prabhu and Bhattacharya (1951) found an average of 22.64 and 17.21 per cent of spermatozoa dead in the first and the second ejaculates, respectively, in buffalo semen.

**Biochemical Attributes.** From the results of a number of investigations by different workers in India, there are indications that besides differences in physical attributes which have already been discussed, there are some important differences in the biochemical properties of the ejaculates of the bull and the buffalo. The findings of these workers have been combined with some data collected by Mann (1954) for comparison and are shown in Table IX.

TABLE IX. DIFFERENCES IN THE BIOCHEMICAL ATTRIBUTES OF BUFFALO AND BULL SEMEN

Attributes	Buffalo			Bull			Statistical significance of differences
	Mean	S.D.	Range	Mean	S.D.	Range	
Total reducing substance (mg. per 100 ml.)	700.40	±52.10		796.59	±42.05		
Initial fructose (mg. per 100 ml.)	837 <sup>a</sup>		375-1440 <sup>a</sup>	610.85	±38.85		*
	355.10	±17.30		775 <sup>1a</sup>			
	718 <sup>2a</sup>			826 <sup>1a</sup>			
	661 <sup>1c</sup>		125-1423 <sup>a</sup>	932.82 <sup>b</sup>			
	782 <sup>a</sup>			552 <sup>a</sup>	±169		
Fructolysis index	1.44	±0.11		1.99	±0.15		†
1st hour							
Total nitrogen (mg. per 100 ml.)	455 <sup>a</sup>		344-625 <sup>a</sup>	736 <sup>d</sup>			
Nonprotein nitrogen (mg. per 100 ml.)	102 <sup>a</sup>		83-140 <sup>a</sup>	45 <sup>d</sup>			
Chloride (mg. per 100 ml.)	373.20	±55.30		247.70	±26.00		†
Calcium (mg. per 100 ml.)	40.50	±2.10		25.00	±3.20		*
	42 <sup>a</sup>		35-62 <sup>a</sup>	34 <sup>d</sup>		24-45	
Ascorbic acid (mg. per 100 ml.)	4.13 <sup>1a</sup>			14.29 <sup>1a</sup>			
Citric acid (mg. per 100 ml.)	419 <sup>a</sup>		322-820 <sup>a</sup>	720 <sup>d</sup>		340-1150	
Total phosphorus (mg. per 100 ml.)	103.20	±8.90 <sup>1a</sup>		47.30	±2.50 <sup>1a</sup>		*
Organic phosphorus (mg. per 100 ml.)	85.20	±7.20 <sup>1a</sup>		41.50	±4.80 <sup>1a</sup>		*
Inorganic phosphorus (mg. per 100 ml.)	65 <sup>1a</sup>		50-60 <sup>a</sup>	73 <sup>d</sup>			
	6.40	±0.60 <sup>1a</sup>		5.00	±0.50 <sup>1a</sup>		
	6.30	±0.40 <sup>1a</sup>		5.60	±0.40 <sup>1a</sup>		
	17 <sup>1a</sup>		14-25 <sup>a</sup>	9 <sup>d</sup>			
Acid-soluble phosphorus (mg. per 100 ml.)	72.20	±3.00 <sup>1a</sup>		29.40	±3.20 <sup>1a</sup>		*
Acid phosphatase activity (Bodansky Unit)	64.30	±3.20 <sup>1a</sup>		27.50	±3.90 <sup>1a</sup>		*
Alkaline phosphatase activity (Bodansky Unit)	305.20	±43.80 <sup>1a</sup>		145.10	±31.40 <sup>1a</sup>		*
	306.80	±41.10 <sup>1a</sup>		167.00	±10.60 <sup>1a</sup>		*
	251.70	±37.20 <sup>1a</sup>		133.80	±14.10 <sup>1a</sup>		†
	266.50	±42.20 <sup>1a</sup>		151.60	±18.5 <sup>1a</sup>		†

Sources: <sup>a</sup> Roy *et al.* (1958) combined with Pal (1957) <sup>b</sup> Luktuke and Bhattacharya (1957) <sup>c</sup> Pal *et al.* (1956) <sup>d</sup> Mann (1954) <sup>e</sup> Ehlers *et al.* (1953) cited from Mann

Ejaculates: <sup>1</sup> first ejaculate <sup>2</sup> second ejaculate

Semen source: <sup>1a</sup> whole semen <sup>1b</sup> seminal plasma

Statistical significance: \* significant at 1% level, † significant at 5% level.

The work of Roy *et al.* (1958) merits particular consideration, for they conducted the investigations on the buffalo and the bull (*Bos taurus* maintained in India) simultaneously and statistically analyzed the data for determining the differences in the two species in biochemical properties of semen.

The differences that can be observed in buffalo and bull semen with respect to the activity of acid and alkaline phosphatases and content of ascorbic acid, calcium, chloride, total and acid-soluble phosphorus, total nitrogen, and nonprotein nitrogen are of con-

siderable interest. Except for an estimate by Pal (1957) on a limited number of samples, the initial fructose content of buffalo semen has been found to be lower than that of bull semen. The initial fructose content of bull semen as reported by Ehlers *et al.* (1953) is, however, lower than the estimates of fructose in buffalo semen barring that of Roy *et al.* (1958).

A final conclusion regarding the differences in the biochemical properties of semen of the two species should not, however, be made at this stage, as the comparisons are based on rather limited observations. Moreover, it should be kept in mind that besides species differences other factors, such as nutrition, management, physiological status of the animals, and climate, may affect some of the properties.

#### SEASONAL VARIATION IN SEMEN QUALITY

Various workers in different countries have observed that along with climatic changes there are seasonal variations in the quality of semen of different species of farm animals.

Seasonal changes in the quality of semen of two Indian breeds of buffaloes have been reported (Malkani, 1954; Kushwaha *et al.*, 1955; Roy *et al.*, 1958). In one investigation by Roy *et al.* (1958) simultaneous observations were made on the seasonal changes in a number of seminal attributes of the buffalo and the bull (Table X).

TABLE X CHARACTERISTICS OF BUFFALO AND BULL SEMEN DURING DIFFERENT SEASONS OF THE YEAR \*

	Winter	Spring	Summer	Rain	Autumn
Sperm density per ml $\times 10^6$	1295.7 $\pm$ 124.7	1563.2 $\pm$ 101.6	1277.4 $\pm$ 117.0	1040.3 $\pm$ 114.7	1098.2 $\pm$ 125.3
Living sperm per ml	1658.8 $\pm$ 65.2	1537.7 $\pm$ 100.7	1458.2 $\pm$ 105.4	1277.7 $\pm$ 119.7	1057.5 $\pm$ 189.9
Percentage of living sperm	89.0 $\pm$ 3.0	62.2 $\pm$ 3.9	63.2 $\pm$ 4.6	79.2 $\pm$ 2.3	82.8 $\pm$ 1.8
Percentage of total abnormal sperm	23.0 $\pm$ 2.1	21.1 $\pm$ 2.5	15.2 $\pm$ 2.2	10.1 $\pm$ 1.1	12.4 $\pm$ 2.0
Percentage of living abnormal sperm	22.5 $\pm$ 2.3	14.0 $\pm$ 1.9	11.6 $\pm$ 2.6	12.7 $\pm$ 2.8	17.8 $\pm$ 3.8
Fructolytic index, 1st hour	1.81 $\pm$ 0.16	1.17 $\pm$ 0.17	1.31 $\pm$ 0.17	1.73 $\pm$ 0.37	1.27 $\pm$ 0.24
	2.09 $\pm$ 0.19	1.86 $\pm$ 0.21	1.64 $\pm$ 0.18	1.66 $\pm$ 0.22	1.81 $\pm$ 0.17

\* Figures in italics indicate observations on *Bos taurus* maintained in India.

There was the common finding by the different workers that the quality of semen in the buffalo was at its best during spring. Earlier

investigations at IVRI revealed that better-quality semen was produced by the bull, the ram, and the goat during winter and spring seasons (November to April) and that there was a decline in the semen quality during summer and autumn (May to October) (Mukherjee and Bhattacharya, 1952a, b; Shukla and Bhattacharya 1952a, b). In the buffalo also there is a decline in semen quality in summer and autumn seasons, as compared with spring, but unlike the bull semen, the buffalo semen does not retain its high quality during winter (Kushwaha *et al.*, 1955; Roy *et al.*, 1958). The decline in the semen quality in both the buffalo and the bull during summer and autumn is possibly attributable to the consequences of the difficult problem of heat dissipation the animals have to face under the stress of hot or warm humid conditions of summer and autumn months.

"The buffalo is sensitive to extremes of cold and heat, and as it is less able to adapt itself to colder climate than cattle" (Kaleff, 1942), it is not surprising that in this species there is a decline in semen quality also during winter. It is generally accepted that the thermo-



Figure 30. This type of Murrah buffalo cow sometimes yields 10,000 lb. or more of milk in a lactation period of nine to ten months, with an average butterfat content of 6 to 7 per cent.



regulatory mechanism of the buffalo is comparatively less efficient than that of the bull.

Malkani (1954) did not find a very marked decline in the semen quality of Surti buffaloes during winter—which is mild in the area where she conducted her investigation.

In seasonal variation of semen quality, besides the problem of thermo-regulation there are indications of involvement of the endocrine system. Bhatnagar *et al.* (1955) have reported that, as in other farm animals, the increased or decreased activity of the testes in the buffalo bull is associated with the increased or decreased activity of the thyroid.

#### DILUTION AND STORAGE OF SEMEN

The phenomenal success of artificial insemination in cattle is in no small measure due to the development of efficient semen diluents like egg yolk-phosphate by Phillips and Lardy (1940) and egg yolk-citrate by Salisbury *et al.* (1941)

Because of the discouraging results with egg yolk-phosphate and egg yolk-citrate for storage of buffalo semen, a group of investigators tried other diluters. Srivastava *et al.* (1953) made phosphate buffer extract of acetone precipitate of egg yolk according to the method of Maycr and Lasley (1945) and preserved buffalo semen in this medium and compared it with a part of the sample diluted in egg yolk-phosphate. The keeping quality of the active principle of the egg was found to be superior to egg yolk-phosphate diluent as judged by the motility of spermatozoa. Srivastava and Prabhu (1956) compared six diluters with regard to their keeping ability of buffalo semen: active principle of egg yolk in phosphate buffer, egg yolk-phosphate, egg yolk-citrate, autoclaved milk, Kampsehmidt's glucose-sodium bicarbonate-egg yolk diluter with addition of sulfamezathine and "Spermasol." Best results were obtained with glucose-sodium bicarbonate diluter followed by "Spermasol" for storage extending over a period of one week, and the percentages of spermatozoa which survived at the end of that period were 32.6 and 28.4, respectively.

For actual insemination work in buffaloes, Gokhale (1958) used egg yolk-phosphate, egg yolk-citrate, and Kampsehmidt's diluter with addition of sulfamezathine. The composition and method of preparation of the last-named buffer are as follows: 4 parts dextrose

solution (5 per cent),  $C_6H_{12}O_6$  (Analar); 1 part sodium bicarbonate solution (1.3 per cent),  $NaHCO_3$  (Analar); 1 part sulfamezathine sodium (1.C 1. brand) solution (2 per cent); and 1 part egg yolk.

The solutions are first prepared separately and autoclaved at 15-lb. pressure for 20 minutes. They are then mixed in the proportions mentioned above and added to the egg yolk; pH is maintained at 6.8.

It was found that though the average time of sperm survival in Kampschmidt's diluter was 180.04 hours, as compared with 199.05 and 157.47 hours in egg yolk-citrate and egg yolk-phosphate, respectively, the motility rating of 3 or above was maintained in this medium for a period (127.2 hours) appreciably longer than in egg yolk-citrate (100.2 hours) or egg yolk-phosphate (77.5 hours). Use of glucose-sodium bicarbonate-sulfamezathine buffer also showed a higher conception rate (52.29 per cent), as compared with egg yolk-citrate (40 per cent) and egg yolk-phosphate (41 per cent). The fertility rates were based on 90 to 120 days non-return.

Roy *et al.* (1955) compared buffalo semen in egg yolk-citrate buffer with or without addition of glycine and noticed that a higher motility rating was maintained in glycine-added diluent and that the conception rate was also slightly higher with its use.



Figure 31. Buffalo calves resulting from artificial insemination in India.

Singh and Tomar (1959) evolved a sodium bicarbonate-glucose-fructose buffer with egg yolk for storage of buffalo semen. Sodium bicarbonate, glucose, and fructose were dissolved in double distilled water to make 1.3 per cent, 5 per cent, and 5 per cent solutions, respectively. The final buffer was made by mixing 10 per cent of the sodium bicarbonate, 40 per cent of the glucose, and 25 per cent of the fructose solutions with 25 per cent of egg yolk. Buffalo semen maintained a motility score of 3 in this buffer for a longer period than in egg yolk-citrate or egg yolk-glycine diluter. Spermatozoa, however, lived for 23 days in egg yolk-glycine diluter, as compared with 12 days in egg yolk-sodium bicarbonate-glucose-fructose buffer.

Veeramani Ayyar (1952) tried boiled milk with citrate buffer as a diluter for bull and buffalo semen and found that semen could be preserved in this medium as effectively as in egg yolk diluter.

The development of a suitable diluent for buffalo semen is a pressing problem. How much biochemical differences account for the poorer keeping quality cannot be stated at present. Only with further research may an answer be found.

**Dilution Rate.** The effect of the rate of dilution on the storage of buffalo semen remains to be explored. For routine insemination at IVRI, the dilution of buffalo semen has not exceeded 1:20 during the course of the last three years, as the demand for buffalo semen has not been very high. No difference in fertility rates has been observed with dilutions ranging from 1:5 to 1:20.

**Antibiotics.** Several investigators have claimed that the addition of antibiotics to the semen diluents improves the keeping quality and fertility of spermatozoa. Gokhale (1958) found that addition of penicillin (500 to 1,000 international units per ml.) and dihydrostreptomycin sulfate (500 to 1,000  $\mu$ g. per ml.) to buffalo semen extended in glucose-sodium bicarbonate-sulfamezathine diluter did not improve the survival time of spermatozoa any more than when semen was diluted with the buffer without addition of antibiotics. The diluting medium with antibiotics, however, maintained a motility of 3 or above for a somewhat longer period (18.2 hours). The use of antibiotics or their omission did not make any difference in the actual fertility rates.

In spite of this finding, the investigation was conducted on a very limited scale, and it may be advisable to use antibiotics in the preservation of buffalo semen until it has been shown from more elaborate,

controlled experiments that the use of these drugs has no beneficial effect. In several artificial insemination centers in India they are being used in buffalo semen, as in bull semen, as a routine measure.

#### DEEP FREEZING OF SEMEN

This method of semen preservation holds much promise for the improvement of buffaloes, since these animals are found in countries where transport facilities are inadequate and distances often vast. Any process by which semen can be preserved over long periods should be of interest in these countries. Unfortunately, however, very little work has so far been done on deep freezing of buffalo semen. This has mainly been due to lack of necessary refrigeration facilities in most of the areas where the buffalo is an important farm animal.

Reports are available of only a few preliminary investigations on preservation of buffalo semen by deep freezing (Bhattacharya and Srivastava, 1955, Roy *et al.*, 1956). From these it appears that no special modification of techniques employed for deep freeze preservation of bull semen is necessary for freezing buffalo semen. Roy *et al.* (1956) used egg yolk medium with glycine for preservation of buffalo semen by deep freezing and noticed that there was a recovery of about 80 per cent of the initially motile spermatozoa when frozen samples were thawed at room temperature. No fertility results of the use of frozen buffalo semen are so far obtainable.

#### INSEMINATION

Insemination of the female buffalo is accomplished by the same techniques as in cows. The rectovaginal method of insemination is being used more and more in India, especially when the inseminator is a veterinarian. The speculum method is more favored by lay technicians. If the rectovaginal technique is used, the manipulation through the rectal wall should be done gently and cautiously, for in the buffalo the capillaries of the rectal wall are more fragile than those in the cow and as a consequence bleeding may result readily.

The need for cleanliness during insemination and the necessity of precautions for using a sterile speculum and inseminating pipette are as essential in the buffalo as in the cow.

There is no authentic information regarding the relative efficacy of insemination in the cervix, body of the uterus, or the horns of the uterus in the buffalo. It is, however, generally presumed that the site of deposition should be the same as in the cow to give similar fertility results in the buffalo.

VanDemark and Hays (1954) have observed that in cows spermatozoa traveled up the Fallopian tubes to the ovarian end in two to four minutes following deposition of semen in the cervix. Rao (1954) found that the speed of sperm transport in the buffalo was about the same as in cows, and spermatozoa reached the anterior third of the Fallopian tubes in three minutes and twenty seconds following artificial insemination. The optimum number of actively motile spermatozoa required per insemination in the buffalo needs to be determined.

#### ESTRUS PHENOMENON

The reproductive physiology of the female buffalo differs from that of the cow in several respects, and it is, therefore, necessary to know the peculiarities of the buffalo to obtain good success with artificial insemination in this species.

In comparison with the cow, the buffalo reaches puberty and sexual maturity at a later age. It has been reported that in Bulgaria the usual breeding age of the buffalo is three years (Kaleff, 1932). Similar reports have been obtained from Malaya, Cambodia, and India (Marsh and Dawson, 1948; Baradat, 1949; Bhattacharya, 1953). Gorbelyk (1935) observed that the buffalo in Azerbaijan reached puberty between two and three years of age, though with good feeding she might do so at one and one-half years of age.

Information from different countries indicates that the duration of estrus is appreciably longer in the buffalo than in the cow and usually lasts 24 hours or more (Kaleff, 1942; Bhattacharya, 1953; Hafez, 1954). In Egypt Hafez (1954) found that the onset of estrus took place between 6 P.M. and 6 A.M. in 84 per cent of the cases observed. MacGregor (1941) has reported that in swamp buffaloes "desire seems to cease with daylight and mating usually occurs only at night." Many buffaloes do not exhibit pronounced signs of estrus, and the incidence of silent heat is more common in the buffalo, so that many heats may be missed unless greatest care is exercised in detection.

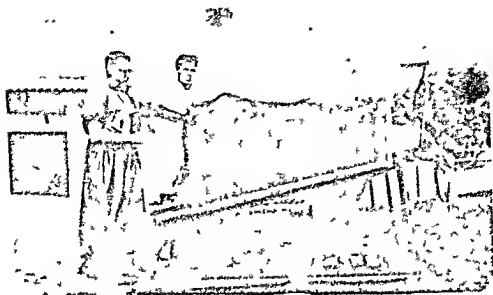


Figure 32 Inseminating the buffalo cow by the rectovaginal technique

The length of the estrous cycle of the buffalo has been reported to be about three weeks by several investigators, whereas some others have observed average cycle lengths of 28 to 30 days. In the Malayan water buffalo heat is said to recur at monthly intervals and to last for two weeks if fertilization does not occur. This, if true, is an unusual type of behavior for Bovidae (Asdell, 1946).

From available information there are reasons to believe that the seasonal variation in the reproductive function of the female buffalo is more pronounced than in the cow. Villegas (1928) reported that in the Philippines high sexual activity is observed in the buffalo during the rainy season and cooler months. In an investigation in Egypt, Hafez (1955) noticed buffaloes remaining completely anestrus during the hot weather (April to July) and returning to sexual activity with the onset of autumn (August). In another investigation Asker and El-Itaby (1958) found that about 75 per cent of the services in buffaloes in that country occur during November to February and not more than 4 per cent of the services occur during summer. Several workers have also reported on seasonal variation in the reproductive function of female buffaloes in India (Arunachalam *et al.*, 1952, Rao and Murari, 1956). In one investigation on buffaloes, some

kept under poor and others under better managerial conditions, De (1957) observed that under both the conditions about 80 per cent of the total number of estrous periods recorded were during October to March and only 20 per cent during April to September. The period of lower sexual activity coincided with higher air temperature and higher humidity.

Very meager information is available on the time of ovulation in the buffalo in relation to the stage of estrus. From an investigation on Murrah buffaloes at IVRI, it has been estimated that ovulation in the buffalo occurs 4 to 30 hours after the end of heat, the average period being 11.4 hours. Rao (1954) observed that the ovum in the buffalo was fertilizable 24 hours before the end of estrus, at the earliest, and 10 hours following the termination of heat, at the latest, when the duration of estrus was 36 hours on an average. He also observed that spermatozoa survived in the reproductive tract of the female buffalo for 36 to 48 hours.

The pronounced seasonal variation of the estrous phenomenon and the greater difficulty in the detection of heat in the buffalo make organization of artificial insemination more difficult in this species.

#### CARE AND MANAGEMENT OF THE SIRE

In several respects, the care and management of the buffalo sire differ from these of the bull. The buffalo bull matures sexually more slowly. Investigators in Asia and Africa have reported that the buffalo bull is put to first service not earlier than three years of age and quite often at three and one-half years or more. There are reports available from Italy and Trans-Caucasus in the U.S.S.R. of buffalo bulls being used much younger—at around two years of age (Maymone, 1942; Agabelli, 1956). MacGregor (1941) states that in the buffalo "by six or seven years there is frequently a loss of potency with a rising proportion of unsuccessful services, but desire continues until he is twelve or more during which time his muscular strength seems to increase. Complete senility, that is loss of muscular strength as well as desire, does not set in till over fifteen. A good buffalo bull of river type can serve 100 cows a year, but it is unusual to allow more than 12 cows to each bull, as each of them will be served several times during her heat. There is no rutting season or periodical masculine desire."

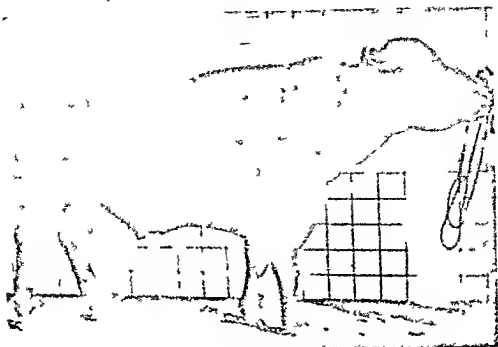


Figure 33 A Murrah buffalo bull

In Italy, buffalo bulls are replaced after four to five years of service (Maymone, 1942) Investigations by El Itrby and Asker (1957) have revealed that in Egypt the average useful life of a buffalo bull is only four and one-half years It appears that the effective breeding life of the buffalo bull is comparatively shorter than that of the bull Consequently, much greater attention and care are necessary for the buffalo sire if the owner is to get the best out of him

Not much information is available on how intensely a buffalo sire should be used for hand mating or how frequently collection of semen should be made for artificial insemination Lazarus (1946) suggests that buffalo bulls should be sparingly used and services so adjusted that the bull does not cover more than 75 times in a year Sayed and Oloufa (1957) reported that semen quality in samples from buffalo bulls with three collections per week was lower for all characteristics than in samples from those with one collection a week On some occasions bulls subjected to three collections per week did not show any libido

Asker and El Itrby (1958) observed that in Egypt as a consequence of the fact that about 75 per cent of the services in buffaloes occur during four months of the year the breeding buffalo bulls are



used at least three times a week for service during the breeding season, and according to the authors such heavy use of the sires may be responsible for production of poor-quality semen and a low conception rate. At IVRI, Murrah buffaloes from which semen has been collected twice a week for a period of three years have shown no detrimental effect.

The shelter requirements of the buffalo are different from those of the bull because of the buffalo's lower heat tolerance. During hot summer months in the tropics buffaloes need special protection from the sun and should have free access to clean wallows. If that cannot be provided, they should have cold showers or good splashing with water daily during the hot weather.

Buffalo bulls are more apt to develop strong antagonism among themselves than dairy or beef bulls, and for this reason more vigilance is necessary to keep them apart. Fights between buffalo bulls are more dangerous and not infrequently end in fatality or serious injury to one or both of the contestants.

The need for attention to good feeding, watering, exercise, sanitation, and disease prevention is the same for the buffalo sire as for other bulls. Buffalo bulls should not be exercised in the sun during hot summer months, and if left loose in the paddock, they should have plenty of tree shade.

In India, there are a number of military dairy farms where large number of buffaloes are very well managed. The young buffalo calves are segregated from the rest of the herd at the age of nine months. At two years of age a nose ring is attached. During this period they are handled, groomed, given sufficient exercise and good feed, individually. In hot weather, they are taken into wallows when these are not occupied by the female stock; or if wallows are not available, they are washed down at 8 A.M. and 6 P.M. During hot weather in the plains the animals are not exposed to the direct rays of the sun between 10 A.M. and 6 P.M. A well-cared-for buffalo bull is used for breeding at the age of two years if well developed. For the first year, he is allowed up to two services per month, and the number of services per bull is gradually increased to four to eight services.

Services are given before feeding, as bulls seem to perform more quickly if this procedure is followed. If the interval between two successive services is long (seven to ten days or more), the bull is

allowed to serve a second time, since the first ejaculate may contain a number of dead spermatozoa

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## Sheep and Goats

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CLAIR E. TERRILL

Artificial insemination of sheep and goats has not passed far beyond the experimental stage in the United States, however, sheep have been artificially inseminated on a huge scale in Russia for many years, and large scale plans have been attempted by individual sheepmen in Australia, Argentina, and many other countries of the world. Sheep can be easily and economically bred artificially, particularly on the Western ranges where they are usually bands of from one to two thousand head, and often several thousand head or more are under one management.

The chief advantage of artificial insemination of sheep or goats lies in the fact that it permits intense selection of sires with exceptional merit, and thus provides the opportunity to exploit fully the value of superior sires. Several hundred offspring are the maximum to be expected from a ram in natural service, whereas several thousands are possible with artificial breeding. Thus, the breeding value of a sire can be extended to at least ten times as many offspring by artificial insemination. The initial investment in rams and the cost of maintenance of rams can be reduced because of the smaller number needed with artificial insemination.

Artificial insemination is advantageous where service from an outstanding male is desired simultaneously in several localities. Fresh semen can be shipped successfully if the time required is not greater than one to three days. However, the per cent of fertility can

be expected to decline with longer storage times. International transport of semen may become more common in areas where air transport facilities are efficient.

Valuable sires which are incapable of service because of age or injury may be utilized through artificial insemination, electrical stimulation being used for the collection of semen. This practice is not indicated if the inability to serve is due to some inherited defect of the sire. Artificial insemination should be used to increase merit, and the possible spreading of undesirable traits has to be guarded against constantly.

The disadvantages of artificial insemination of sheep must be clearly recognized. Sires that have not been thoroughly proved may spread undesirable traits much more widely through artificial insemination than by natural mating. This is particularly true of recessive defects, which may not show up under ordinary use.

Artificial insemination programs must be carefully and skillfully conducted or lower fertility rates may result, and to be most successful they must be limited to rams of above average fertility. Any failure to settle the ewes promptly will result in a prolonged lambing season and lowered uniformity of the lamb-crop. Perhaps the greatest disadvantage of artificial insemination of sheep is its expense. Kelley *et al.* (1942) state that the limiting factor in Australia will be cost. To be practical, the monetary gain from its use must at least balance the added cost in artificially breeding the ewes.

Considerably more work has been done on artificial insemination with sheep than with goats. However, much of the material presented applies to goats as well as to sheep, and an increasing amount of information is becoming available on artificial insemination of goats.

#### MANAGEMENT OF RAMS

Rams to be used for artificial insemination must be in a condition of high fertility. Rams of fairly low fertility might settle a small flock of ewes in a period of 60 days under natural mating, but might partially fail when artificially mated to a large number of ewes. A highly fertile ram will produce larger numbers of live, normal sperm than will a ram of low fertility. His semen can not only be used to inseminate larger numbers of ewes per day but also it will probably produce a higher percentage of pregnancies.

The rams should be well fed for at least a month prior to the breeding season. An ample supply of green pasture or alfalfa hay, plus some grain or protein supplement, with access to salt, water, and minerals, if necessary, should be sufficient. Moderate exercise should be encouraged. Rams should be kept in a thrifty condition but should not be allowed to become overfat. Those that have been highly fitted for show or sale should be shorn and turned out to pasture for at least several weeks before use. Rams will do better in small bunches and should have access to shade or cool quarters, particularly in a warm climate.

#### SEMEN COLLECTION

Three methods have been used most successfully for collecting semen from rams. The simplest method is to allow the ram to serve the ewe and then collect semen from the vagina, this requires practically no equipment and is usually less difficult than with the artificial vagina. The use of an artificial vagina with a dummy ewe eliminates the necessity of maintaining ewes for the collection of semen and minimizes the possibilities of contaminating the semen. Collecting semen from the ram by means of electrical stimulation is the least time-consuming, and is not dependent on the sexual desire of the ram.

In comparison of semen obtained by the three methods (Terrill, 1940) semen of similar quality was obtained from the vagina of the ewe and the artificial vagina. Semen obtained with the artificial vagina was slightly lower in volume and higher in concentration of spermatozoa than that obtained from the ewe. Semen obtained by electrical stimulation was of much thinner consistency, and the concentration of spermatozoa was much lower than with the other two methods. The total number of spermatozoa obtained at each collection was much more variable with electrical stimulation than with the other two methods. Results of artificial insemination with both fresh and stored semen gave 61 per cent pregnancies with semen collected from the vagina of the ewe, 52 per cent from electrical stimulation, and 42 per cent from the artificial vagina. All three methods of semen collection have been used successfully with goats and the advantages and disadvantages of each method appear to be similar to those with sheep. Collection with the artificial vagina appears to be the most common method with goats.



*From the Vagina of the Ewe.* Collection of semen from the vagina of a ewe is best done with a ewe out of heat because the vagina is more apt to be dry and free from mucus. The ewe should be firmly tied in a stanchion, and any fluid in the vagina should be removed before service. The semen should be removed immediately after each service with a pipette (shown in Figure 34). Care should be taken to remove all of the semen and to avoid contamination with urine or other foreign material. Repeated collections can be made from a single ewe, but a different ewe should be used for each ram to avoid uncertain parenthood. Rams which refuse to serve a ewe out of heat can usually be trained by repeated trials. They can often be induced to serve a ewe out of heat if they are first allowed several services with a ewe in heat and then switched to a ewe out of heat. All collections should be made in the same place; and rough handling, excitement, and undue noise should be avoided.

The interval between services will vary among rams from about five minutes to an hour or longer. In general, services should not

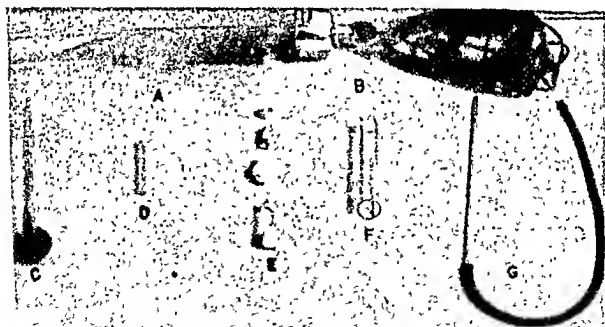


Figure 34. Equipment for artificial insemination of sheep. A. Artificial vagina assembled for use. B. Head lamp. C. Semen-collecting pipette for removing semen from ewe, made from glass tubing about 12 inches long and  $\frac{3}{8}$  inch in diameter, rounded at the ends and equipped with a 15 to 25-cc. rubber bulb. D. 5-cc. graduated test tube for collecting semen. E. Small vials for storage or shipment of semen. F. Vaginal speculum, made from pyrex glass tubing, 6 inches by  $\frac{3}{8}$  inch (should be slightly shorter for goats). G. Inseminating pipette consisting of a 1-cc. pipette graduated to the tip and mouth tube (a rubber bulb may be substituted for the latter). Equipment suitable for sheep can also be used for goats. (Benediktovic, 1934; Palayceva and Famenka, 1936.)

be permitted more often than every 15 minutes, and from three to eight ejaculates may be obtained in a half-day McKenzie and Berliner (1937) reported that rams produced from 13 to 24 ejaculates in nine hours, with some decrease in number of sperm with successive ejaculates Chang (1945) found that total sperm output increased with the number of collections up to six per day There was some decrease in volume and concentration of each ejaculate with an increase in the number of collections, but no change in quality, as shown by percentage of abnormal sperm and keeping quality However, Swanson and Blackwell (1955) found that collection of one ejaculate per day was superior to four per day in both quality and total number of sperm obtained A continuation of this study (Harrington *et al*, 1956) showed that collections of two ejaculates and one ejaculate per day and one ejaculate every other day ranked in that order in total number of sperm produced per day Motility was slightly higher for collections made every other day

**With an Artificial Vagina** An artificial vagina, as shown in Figure 34 can be of very simple construction, and can be used with either sheep or goats It consists of a metal or heavy rubber outer tube and a thin rubber inner tube surrounded by a layer of warm water and air The outer tube should be about  $7\frac{1}{4}$  inches long and 2 inches in diameter One or two petcocks in the outer tube are used for filling it with warm water and for adjusting the air content to obtain moderate pressure The inner tubing may be made from  $1\frac{1}{2}$  inch band tubing 0 031 gauge, and should extend at least one inch and not more than two inches beyond the ends of the outer tube so that it can be folded back over each end and held in place with rubber bands A rubber funnel is attached to one end, leading to a small test tube to catch the semen A graduated 5-c c test tube is convenient if a record of semen volume is to be made The open end of the artificial vagina should be well lubricated Mineral oil, vaseline, or gum tragacanth may be used for a lubricant \*

A more elaborate artificial vagina designed by Millar (1958) appears to be more durable and easier to clean or sterilize, and provides

Lambert and McKenzie (1940) recommended the following lubricant for use with artificial insemination Mix thoroughly 6 gm of powdered gum tragacanth with 10 c c of glycerine Add to this mixture, stirring continuously 100 c c of water Keep in refrigerator to prevent molding

better protection against temperature shock and loss of the ejaculate.

The temperature of the artificial vagina is very important. The ram may refuse to serve if it is too hot or cold, and it is sometimes difficult to induce a ram to serve again after an unsatisfactory experience. The temperature within the inner tube at time of service should be between  $41^{\circ}$  and  $44^{\circ}$  C. ( $106^{\circ}$  to  $111^{\circ}$  F.). Some decrease in temperature between the time of preparation and service must be allowed for. A convenient arrangement for filling and warming the artificial vagina consists of a hot plate on a high shelf and a water container with an outlet at the base with rubber tube and stop clamp. The water can be kept at  $80^{\circ}$  to  $90^{\circ}$  C., and the artificial vagina can be quickly warmed to the desired temperature by allowing the hot water to pass through it.

The artificial vagina either can be used with a dummy ewe (Figure 35), or it may be held in the hand beside a live ewe.

*By Electrical Stimulation.* A method of electrical stimulation in rams was developed by Gunn (1936). A modification involving the use of a single rectal bipolar electrode was made by Laplaud and



Figure 35 Dummy ewe used for collection of semen at the United States Sheep Experiment Station, Dubois, Idaho. Construction is simple, and rubber bands are used to hold the artificial vagina in place (Courtesy United States Department of Agriculture)

Cassou (1945) The use of the bipolar electrode with the gradual raising and lowering of the current greatly reduces general body reaction and appears to be generally as satisfactory in producing an ejaculate as Gunn's method. A number of workers have adapted and used such equipment for rams and goats, including Blackshaw (1954a), Dziuk *et al* (1954), Edgar (1957), and Barker (1958).

The equipment in Figure 36 is very satisfactory, although partially homemade, as described by Dziuk *et al* (1954). More elaborate equipment can be obtained. The source of power is 110 volt, 60 cycle and is reduced to 30 volts by a transformer, which also isolates the electrodes from the line source. The rectal probe is made of rubber hose about  $\frac{1}{4}$  inch in diameter with solder rings one inch apart. Alternate rings or electrodes are connected to provide a difference in electrical potential between adjacent electrodes.

The method can be used with the ram held standing as shown in Figure 36, but it is sometimes desirable to restrain the animal in an extended position. The probe is lubricated, moistened, and inserted so the last ring is just inside the anus. The penis may be extruded before stimulation but it will generally protrude with stimulation. The filiform appendix and the end of the penis should be held in a test tube or small vial. Voltage is gradually increased and then reduced to zero during a five second period followed by an equal rest period. The stimulus is gradually increased until semen is obtained in the order of 2, 5, and 8-volt peaks. Considerable variation can be expected in the number of stimuli necessary to produce semen and in the volume of semen and concentration of sperm obtained.

#### EXAMINATION OF SEMEN

Success with artificial insemination is partly dependent on the quality of the semen used, and this should be checked constantly. This also serves as a guide to the management of the rams, although in some cases deficiencies of the semen cannot be corrected by improved management. It is important to remember that the inseminating dose of semen should be based on the number of viable spermatozoa introduced rather than the volume of semen, and careful examination of each lot of semen (a number of ejaculates from one ram may be combined into one lot to simplify examination) should be made for this reason. With some experience it is possible



Figure 36. Technique for collection of ram semen by electrical stimulation at the United States Sheep Experiment Station, Dubois, Idaho. Above: Method of restraining the ram during collection. Below: Electrical stimulation apparatus. Note the variable transformer, voltmeter, and ammeter, at left, and the rectal bipolar probe, at right. (Courtesy United States Department of Agriculture.)

to predict the fertilizing capacity of semen with fair accuracy This is helpful, particularly if a choice of rams is possible Sterile rams or those with low fertility can usually be detected However, rams with acceptable semen quality sometimes fall in this latter group

Wiggins *et al* (1953) found that libido as measured by the frequency of ejaculation had a significant relationship to fertility as judged by percentage of ewes lambing Of the various semen characteristics studied—including viscosity, pH, volume of semen, motility score, estimated percentage of motile sperm, estimated motility count, total sperm, percentage of normal sperm, percentage of abnormal heads, percentage of live sperm, and percentage of live normal sperm—only volume of semen, estimated motility count, percentage of normal sperm, percentage of abnormal heads, and percentage of live, normal sperm were significantly correlated with the percentage of ewes lambing from normal service Furthermore, in no case was the predictive value high It appears that no single laboratory test with high predictive value of potential fertility is available at present and that success in predicting fertility must depend on a number of traits

A rough appraisal of ram semen, which is often sufficient for practical purposes, can be made without equipment Good ram semen has a creamy appearance and consistency The swirling motion which accompanies high motility can be observed with the naked eye Semen containing a high concentration, but mostly of dead spermatozoa, has a brownish yellow appearance High concentration is indicated by a rich creamy appearance, and as the concentration of spermatozoa decreases the semen becomes thinner and milky or watery in appearance

Records of the characteristics of semen used for artificial insemination can easily be kept and should be available for calibrating dosage and in appraising results These records should include the volume collected, appearance, reaction to litmus or other test paper, estimation of motility, determination of concentration, and an appraisal of abnormal types present These records can be taken by an experienced operator in about ten minutes for a lot of semen if only a rough examination of abnormal types is made A more thorough check on abnormalities may be made periodically

Staining with eosin B in combination with a background stain such as opal blue (Lasley *et al*, 1942) or fast green, FCF (Mayer

*et al.*, 1951), appears to be useful for estimating the proportion of live spermatozoa present and also for classifying abnormal types. A variety of other background stains have been used for ram and goat sperm, as described by Rao (1951, 1956, 1957) and Blackshaw (1958a).

In staining by the opal blue-eosin or fast green-eosin method, a small drop of stain mixture is placed on a clean slide. A glass stirring rod is then dipped in the semen sample, and the amount of semen adhering to it is immediately mixed with the stain on the slide. The flat surface of another clean slide is placed over the mixture, which causes it to spread out in a thin film between the surfaces of the two slides. The slides are then drawn apart without applying pressure and should be quickly dried on a warm plate at 40° C.

The recording of volume is facilitated by collecting the semen in a graduated test tube. Motility should be estimated by a grade or score under the microscope. This estimation may be made at a constant temperature by means of a small hanging drop of semen in a micro stage incubator. Concentration may be measured with a hemocytometer or more rapidly and accurately with a photoelectric colorimeter, as shown by Emik and Sidwell (1947a). Matching the semen with calibrated standards provides a less accurate but more rapid estimate of concentration.

#### SEMEN CHARACTERISTICS

An average ram ejaculate consists of slightly less than 1 c.c. of semen with a creamy appearance. It contains from 2 to 3 billion spermatozoa, of which about 90 per cent are alive. Semen with high concentrations is usually slightly acid in reaction, while that with low concentrations is slightly alkaline. An alkaline reaction of the semen is often associated with poor quality and low fertility. The motility is characterized by a swirling motion so rapid that it is difficult to distinguish individual sperm. Normal spermatozoa are shown in Figure 37, and some abnormal forms are shown in Figure 38. From 5 to 15 per cent of the spermatozoa are abnormal in morphology. Greater proportions of abnormal sperm may indicate low fertility. Most common forms of abnormal sperm are tailless and misshapen and have tapering heads, enlarged middle pieces, and



Figure 37 Normal ram spermatozoon (Courtesy United States Department of Agriculture)

adhered coiled, or bent tails Head abnormalities are most likely to indicate low fertility

The characteristics of goat semen are very similar to those of ram semen as shown by observations on goat semen by Polovceva and Gomenko (1936) Mockel (1937), Phillips *et al* (1943a), Shukla and Bhattacharya (1949 1952), Rao and Berry (1950), Eaton and Simmons (1952), and Dussardier and Szumowski (1952)

Ejaculates of ram semen with less than 1 billion sperm per c c , with a pH of 8.0 or higher, with slow, sluggish motility, and/or with more than 25 per cent abnormal sperm or more than 1 per cent abnormal sperm heads are of doubtful fertility and should not be depended upon for artificial insemination Similar limits will apply to goat semen as shown by Wagenaar (1946) Several ejaculates should be examined over a period of two weeks or more before a ram is judged unfit Some rams improve with use, and some decline Often rams produce poor semen after a prolonged period of sexual inactivity



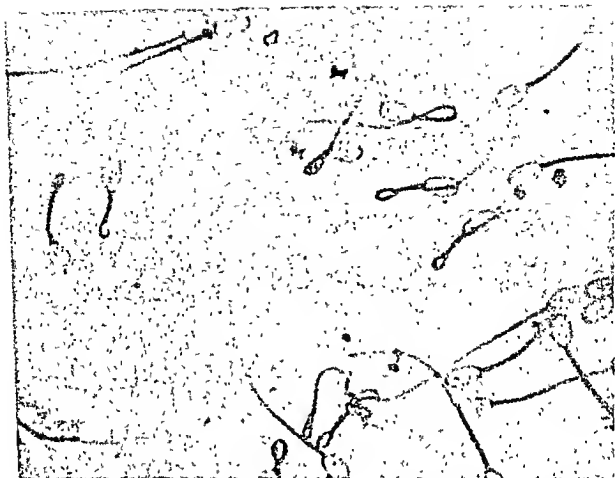


Figure 38. Abnormal ram spermatozoa. Above: Adhered tails. Below: Tailless heads.  
(Courtesy United States Department of Agriculture.)

## STORAGE AND SHIPMENT OF SEMEN

Ram or goat semen has been stored successfully for fertile insemination up to seven days (Green and Winters, Yoshioka *et al*, 1951) Lardy *et al* (1945) state that ram spermatozoa retain motility during storage longer than spermatozoa from other domestic animals. However, lowered fertility often results even from 24 hours' storage, and it is desirable to use fresh semen. This should be convenient where sheep are kept in large bands and inseminations are made regularly. Phillips *et al* (1940) shipped ram semen by air express 2,600 miles. Some lambs resulting from these shipments are shown in Figure 39.

Various temperatures from 0° to 10° C have been recommended for storage and shipment of semen. Temperatures near 0° C (35° F) seem to be optimum for ram semen, as shown by Moore *et al* (1940). Diluted semen may be stored more successfully at higher temperatures, but difficulty with bacterial action may be encountered. Storage of ram semen at 20° C in the presence of CO<sub>2</sub> may be preferable (Blackshaw, 1958b).

The procedure generally adopted by Phillips *et al* (1940) for shipment of semen proved to be satisfactory. The semen was placed in small vials under mineral oil. These vials were corked, wrapped in cotton and placed in larger vials (see Figure 34). Vacuum bottles were partially filled with cracked ice and the vials were wrapped in cotton above the ice. The amount of ice should be varied with the time required in shipment.

Semen must be handled carefully if the sperm are to remain viable. Chang and Walton (1940) have shown that sudden temperature changes are harmful and must be avoided. Diluters and containers should be the same temperature as the semen to be added. Absolute cleanliness is essential. Semen should be cooled gradually for storage although Blackshaw has shown that the presence of egg yolk in the diluent will largely prevent cold shock.

Storage of ram semen after freezing at low temperatures has not been so successful as storage of frozen bull semen (Emmens and Blackshaw, 1955). Fertile inseminations from frozen semen were made by Smirnov (1951). White *et al* (1954) found that ram semen showed very little metabolic activity after freezing, although this

was improved by egg yolk. Graça Araujo (1955) successfully stored frozen ram semen up to 45 days, although the rate of conception was low. First *et al.* (1957) obtained 17 per cent fertility with insemination from frozen ram semen diluted with milk, 7 per cent glycerol, and 1.25 per cent arabinose, as compared with 64 per cent fertility with 0.2 ml. of fresh undiluted semen. Kuznetsov (1956) reported an average fertility of 33.5 per cent with frozen semen. Smith and Polge (1950) found good motility after thawing frozen goat semen.

#### DILUTION OF SEMEN

Dilution of the semen for storage appears to be beneficial in some cases. Minnesota workers (Green and Winters) have found that sheep sperm are preserved longer if an equal quantity of the following solution is added to the semen:  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , 15.4 gm.;  $\text{KH}_2\text{PO}_4$ , 3.2 gm.; sterile distilled water, 1 l.;  $\text{CaSO}_4$ , saturate. The pH should be about 7.0. Workers in Peru have found dilution to be essential for storage at high altitudes.\* However, Keast and Morley (1949) obtained conception rates of 60 and 69 per cent with undiluted semen, as compared with 47 and 56 per cent with diluted semen.

Dilution of ram semen not only is beneficial for storage but is convenient for reducing the concentration so that an ejaculate can more easily be divided into a large number of doses. Kuznetsov (1934) recommended that the number of ram sperm introduced into the cervix should not fall below 50 million. Milovanov (1934) reported that at least 500 million ram sperm were essential for vaginal insemination. Where convenient, it is desirable to allow a greater margin of safety. Panyseva (1940) found a direct correlation between the number of sperm introduced and conception rate ( $r = 0.8483 \pm 0.0777$ ). An ejaculate of 1 c.c. containing 3 billion spermatozoa diluted 1:9 and inseminated in 0.2 c.c. doses permits 50 ewes to be inseminated in the cervix with 60 million sperm each. Motility of ram sperm is greatly reduced or lost at relatively high dilutions of 20 million to 0.06 million per ml. (Blackshaw, 1953).

\* Personal communication with Mr. F. Accame regarding the work of Dr. Mauricio San Martín of the National Institute of Andean Biology, Huancayo, Peru. In general, he found the egg yolk buffer mixture to be very satisfactory. This was confirmed by Easley (1951).

The egg yolk buffer mixture developed by Lardy and Phillips (1939) has been a satisfactory diluent for ram semen. Equal volumes of fresh egg yolk and sterilized phosphate buffer (0.2 gm  $\text{KH}_2\text{PO}_4$  and 2.0 gm  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  per 100 c.c.) are mixed thoroughly by shaking. The pH of the mixture will be very close to 6.75, if not it should be adjusted by the addition of sterile M/15 buffer, described above. Citrate or bicarbonate can be used to replace the phosphate buffer without effect on the glycolysis (Moore, 1949). Moore also reports that sulfathiazole does not affect glycolysis and can be employed to control microorganisms. The replacement of the citrate buffer with glycine has improved the survival of ram semen (Ahmed, 1955, Roy *et al*, 1956, and Skolovskaya *et al*, 1956).

Yoshioka *et al* (1951) used two diluters for sheep or goat semen, each of which increased the conception rate from 56 to 65 per cent. One diluter consisted of 2 parts of 2 per cent boric acid ( $\text{H}_3\text{BO}_3$ ) by volume with 1 part of 1 per cent sodium bicarbonate ( $\text{NaHCO}_3$ ). This was added to the semen in the ratio of 1:3. The other diluter consisted of equal volumes of 0.3 per cent sodium sulfamerazine and 0.2 per cent homosulfamine. This was dissolved in 5 per cent sodium citrate, and the solution was added to the semen in the ratio of 1:1.

Milk appears to be useful as a diluent for ram semen, as shown by Istvan (1956), Filimon *et al* (1956), Macpherson (1957), and Hill *et al* (1958).

#### MANAGEMENT OF EWES

The most difficult practical problem to solve in artificial insemination of sheep is the managing of a large number of ewes under normal feed conditions while allowing the detection and sorting of ewes in heat at a central concentration point. This would be fairly simple under feed lot or farm flock conditions, but it is quite complicated on the range, where tramping and overgrazing near corrals must be avoided. Fortunately, ewes are bred in the fall, when the range forage is least susceptible to injury. The best solution seems to be with the use of temporary corrals which can be readily moved from place to place.

Sheep as well as goats generally have restricted breeding seasons. Artificial insemination will be most successful at the height of the breeding season, which usually falls between September and De-



Figure 39. Carriedale ewes and their lambs at Beltsville, Maryland. These lambs were sired by rams at Dubois, Idaho, through artificial insemination. (Courtesy United States Department of Agriculture.)

cember in the northern hemisphere (McKenzie and Terrill, 1937; Phillips *et al.*, 1943a, b).

Ewes in heat can be detected only by actual trial with a teaser ram. Ewes in heat will stand and allow the ram to mount. Rams with aprons to prevent service may be used as teasers, but vasectomized rams are probably more satisfactory. Even then, it may be necessary to prevent service with an apron part of the time to maintain the sexual aggressiveness of the ram.

Teaser rams may be painted on the brisket and allowed to run with the ewes at all times. Wool grease mixed with powdered paint pigment makes a highly satisfactory raddle or paint. Application on alternate days is adequate (Kelley *et al.*, 1942). Jackets may be used to hold the paint, as illustrated by Barreto and Filho (1944). Marking harnesses holding colored crayon can be used for marking ewes although some inaccuracy can be expected. The rams will mount any ewes in heat and leave paint marks on the rumps of these ewes. Then they can be sorted out by running the hand through a cutting

chute Kelley *et al* (1942) state that those ewes which have been served can be readily separated from those that have been mounted speculatively. Some ewes may not be marked until after they have been in heat for several hours, as rams normally show some partiality toward individual ewes. Some ewes in heat tend to follow the ram and thus escape his attention for a while. However, Kelley (1937) found a low percentage of error in detecting ewes in heat by this method.

Another method of detecting ewes in heat, which may be more thorough but requires more labor, is to keep the teaser rams at the corral and systematically tease the ewes in groups of 50 to 100 or more. Kelley *et al* (1942) have teased about 250 ewes with 10 vasectomized rams in a yard 25 by 40 feet. This packed the ewes loosely enough to let the teasers work through them but closely enough to enable the ewes in heat to be caught easily. The teasers had generally found all of the ewes in heat in about 45 minutes.

Ewes in heat should be removed as quickly as they are detected. The teaser rams should be selected for their aggressiveness, and sterile service should be allowed occasionally. Teaser rams can be broken to lead and can often be trained to follow the observer in and out of the pens. A combination of the two methods of checking ewes for estrus would seem most efficient. Some teaser rams could be run with the band at all times. Ewes which were paint marked could be sorted out twice daily, and a rapid check could be made of the remainder of the flock, using fresh teasers. Ewes which have been served, as indicated by the paint mark, should be inseminated, even though they may be out of heat by the time of insemination. Kelley *et al* (1942) have found that some of these ewes will become pregnant from insemination after the end of heat.

#### INSEMINATION OF EWES

The process of insemination is very simple and with proper organization can be quickly accomplished. Necessary equipment illustrated in Figure 34 consists of a speculum, head lamp, and a syringe or pipette. Various specula may be used. A piece of pyrex glass tubing about  $\frac{7}{8}$  inch in diameter and 6 inches long with polished edges is very satisfactory. It is inexpensive and is easily cleaned. A small flashlight or speculum light may be used instead of the head

lamp. An ordinary 1 c.c. pipette graduated to the tip with mouth tube or rubber bulb is very convenient for insemination. It must be filled with the correct amount of semen for each ewe. It is desirable to use a clean pipette for each insemination. Any rough edges on the tip should be carefully smoothed. Other useful devices have been developed for introducing the semen in the cervix, such as the one described by Robinson (1956).

A special crate or breeding stall may be constructed to hold the ewe for insemination. Minnesota workers (Green and Winters) have devised a simple crate for small numbers of ewes which holds the ewe firmly and permits the rear quarters to be elevated. Quinlan *et al.* (1941) have found that the method of holding a ewe on the ground, partly on her side and back, with the front and hind legs pulled together, was less laborious and equally effective. Argentine workers (Ross, 1942) have devised a revolving platform with three crates so that three men work simultaneously: While one man is putting a ewe in a crate, another is inseminating, and the third is taking a ewe off. This method appears to be very efficient, particularly for large-scale operations. Mr. Ross reports that it is possible to inseminate 110 ewes per hour. To eliminate stooping, the crates may be elevated, or a pit may be dug for the operator. Similar equipment for inseminating sheep is illustrated by Barreto and Filho (1944).

In the process of insemination the pipette should first be filled with the correct amount of semen. The speculum should be lubricated with oil, vaseline, or gum tragacanth and carefully inserted into the vagina. By observing with the head lamp and manipulating the speculum the cervix may be located. It is often turned to one side and must be straightened by manipulating the speculum. The pipette is then introduced through the speculum and is inserted into the cervix. It is usually not possible for the pipette to enter the cervix more than one-eighth to one-quarter inch. The semen may then be slowly and gently expelled into the cervix.

The amount of semen to be used for insemination must be governed by several factors. Only a very small volume should be introduced into the cervix, as large amounts may cause pain and are likely to overflow into the vagina. A volume of 0.05 to 0.2 c.c. has been found to be practical for the ewe. This amount should contain a minimum of 50 to 100 million sperm. Normal undiluted ram semen should contain about 150 million sperm per 0.05 c.c. The number

of sperm actually introduced above the minimum could vary according to the amount of semen collected and the number of ewes in heat at any given time. A minimum as low as 5 million is reported as having been fairly successful in Uruguay.

The designation, if available for each ewe, should be recorded along with the date of service and identification of the sire, if more than one is used. These data should be useful as breeding records, in addition to allowing checks on the success and efficiency of the operations. Large scale operations may be facilitated by branding each ewe with the date or symbol indicating the date of insemination (Cummings 1939). Small number brands made with wool branding paint are satisfactory. These ewes may be run in separate bands for about 12 days after insemination, thus reducing the work of teasing and also cutting down the size of the band which must be trailed to the corral. After the first 12 to 14 days, each successive daily group can be sorted back into the band which is brought in for teasing. Ewes which do not return in heat within 36 to 40 days may generally be considered pregnant.

The estrual cycle of the ewe is normally about 17 days in length, with from two thirds to three-quarters of the cycles falling within 16 to 18 days and only about 10 per cent falling outside the range of 14 to 19 days. From 5 to 10 per cent of the ewes in a band may be expected to come in heat in each of the first 14 to 16 days, provided inseminations are not commenced before the breeding season is well under way. The greatest number of ewes in heat can be expected on the first day, as many ewes will be sorted out that have already been in heat for some time. A sharp reduction in the number of ewes in heat each day will be noted after 14 to 16 days, unless many inseminated ewes return in heat. When fertility is low, ewes, which later prove to be nonpregnant, may fail to return in heat at the end of the first cycle. If large numbers are to be inseminated, the bands may be staggered at 17 to 20-day intervals. Also, this is often necessary to facilitate work at lambing time so that a small crew can work over a longer period of time.

Ewes normally remain in heat about 30 hours although the duration may vary from a few hours up to three days or longer. The time of ovulation in relation to the onset of heat may vary considerably. McKenzie and Terrill (1937) found that ovulation in the ewe generally takes place near the end of estrus. Inseminations made after



the end of heat are less likely to be successful than those made just before the end of heat. Therefore inseminations must be made fairly early in the heat period, or ewes with the short heat periods will be missed. The optimum time of insemination would appear to be just before ovulation or near the end of the heat period. Therefore if all ewes are to be inseminated at least once and also at the optimum time during heat, it is necessary to inseminate at intervals.

It has been generally observed that if more inseminations are made per heat period, a higher degree of fertility results. However, it may be more important to obtain a large number of pregnancies per season from one outstanding ram than to obtain a high percentage of pregnancies in a limited time. It may be advantageous during the first 14 to 16 days of the breeding season to inseminate each ewe only once or not more than once each day she remains in heat. Then on the second round, when fewer ewes are coming in heat, each ewe may be inseminated more often. It would hardly be practical to inseminate more often than twice daily as long as a ewe remains in heat, and it may be impractical to inseminate any ewe more than three times in any one heat period. If ewes are to be inseminated only once in the heat period, a safe plan is to inseminate about 12 to 24 hours after they are first observed in heat. In practice, it is desirable to allow for considerable flexibility above the minimum requirements, both in the number of inseminations per heat period and in the number of sperm per insemination, depending on variations in the number of ewes in heat, length of the heat periods, and the number of spermatozoa collected.

The estrual cycle of the doe is longer than that of the ewe and generally more variable. Studies made by Arriola (1936), Polovceva and Fomenko (1936), Phillips *et al.* (1943b), Barretto and Filho (1945), and Fugazzotto (1947) show average cycle lengths as short as 8 days, with the majority reporting averages from 19 to 23 days. Duration of estrus in the doe is reported by the same workers as ranging from 18 hours to 4 days.

#### PLAN FOR ARTIFICIAL INSEMINATION OF SHEEP

A crew of three men, including one professional worker, either a veterinarian or animal husbandman and two laborers should be able to handle about 1,000 ewes at a time. Facilities should include

a corral for sorting a band of this size, with a large chute for teasing, holding pens for a maximum of about 200 ewes (in heat), 20 teaser rams, and one stud ram and one or more reserve stud rams. Means for collecting semen and a small shed, tent, or trailer for laboratory equipment will also be needed. Inseminating crates arranged on a 'merry go round' device with connecting chutes may be advisable, although the method of holding a ewe on the ground or on a low table might be equally efficient and would not require any special equipment.

The professional worker will collect, examine, and dilute semen while the laborers are checking and sorting out the ewes in heat. This will require from two to three hours. The first morning all ewes in heat will be inseminated, and after that only those ewes that were checked in heat the preceding half-day or earlier and which remained in heat will be inseminated. The number of ewes to inseminate in any half day will vary from about 50 to 125. It will be necessary to collect at least three ejaculates and perhaps more. From 5 to 10 billion spermatozoa will be needed.

The insemination of the ewes will require from one to two hours and may be carried out after the remainder of the sheep have gone out to feed. One laborer places a ewe in the crate and the other takes one out, while the professional worker is inseminating a ewe and recording her number. If the method of holding the ewes by hand is adopted, one laborer will be bringing up the next ewe while the other man is holding the ewe being inseminated. Each ewe should be branded to indicate the date of her first insemination.

The entire procedure will be repeated twice daily, except that in the afternoon the collection of semen and the insemination will be completed before the ewes are brought in for teasing, since the ewes sorted out in the evening will be inseminated first the following morning. It is advisable to allow a rest period during the middle of the day so that collections and inseminations can be spaced as near to 12 hours apart as possible, depending on the length of day.

If several thousand ewes are to be inseminated from the stud ram, it may be desirable to inseminate each ewe at only one heat period rather than to continue insemination through other estrual cycles. Thus, after about each 17 to 20 days an additional 1,000 ewes would be added to the group being teased, and all ewes already inseminated would be cut into separate bands. Rams should be turned

with these ewes to breed any which failed to become pregnant from the insemination.

#### RESULTS FROM ARTIFICIAL INSEMINATION OF SHEEP

Published results from experiments with artificial insemination of sheep range from complete failure to nearly complete success. Insemination at only one heat period per ewe with fresh semen has resulted in from 30 to 75 per cent of pregnancies. This can be increased by insemination at successive heat periods. Quinlan *et al.* (1941) obtained 95 per cent pregnancies using fresh semen at three successive heat periods. Anderson (1945) gives results of insemination of more than 11,000 sheep on farms in Kenya, with an average of 76 per cent of the ewes lambing. A 75 per cent lambing from natural service is considered satisfactory for Merino sheep in Kenya. However, Anderson (1941) has noted that many ewes that do not conceive from artificial insemination also do not return in heat within 17 to 19 days. Gutierrez Fabre (1948) reports conception rates from 60 to 82 per cent from artificial insemination of 38,000 ewes in Uruguay. Natural mating of range sheep in the United States usually results in 85 to 95 per cent of pregnancies after a 25- to 60-day breeding period. It is not expected that artificial insemination will show a higher rate of fertility than natural mating unless the method of natural mating is deficient in some manner.

High fertility from artificial insemination of goats has been reported, with conception rates ranging from 92 to 97 per cent (Rosenberger, 1944; Wagner, 1949; Schmidt *et al.*, 1950), although Blokhuis (1957) obtained 48 to 51 per cent pregnancies with single inseminations. Guha *et al.* (1951) obtained 78 per cent pregnancies from the first insemination, 7 per cent by a second, and 6 per cent by a third. Setinski (1956) obtained a conception rate of 55.5 per cent to the first insemination of goats and an over-all rate of 76.1 per cent. At the A. I. Center near Rouille, France, 700 goats were serviced artificially in 1961 and 1962, with a kidding rate of 65 per cent (Dziuk, 1962).

Kuznetsov (1956) reported that over 28 million sheep were inseminated in the U.S.S.R. in 1955. About 45 per cent of the sheep on collective farms were artificially inseminated.

Published work has demonstrated that if artificial insemination of

sheep and goats is carefully conducted under favorable conditions, the results will compare satisfactorily with those from natural mating under similar conditions

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## Horses and Jackstock

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VICTOR BERLINER

Although artificial insemination has gained its greatest development and economic importance through its utilization in the breeding of dairy cattle, the first systematic exploitation of this breeding method took place with another class of livestock, namely horses. It was the work of the Russian physiologist E. I. Ivanoff, at Russian government stud farms at the beginning of this century, that directed the attention of the public and the scientific world to the possibilities of this new breeding system. Crude as his methods were, compared with the ones used today, his work with horses has to be considered the pathbreaking pioneer work that finally led to the high accomplishments of artificial insemination of today. The stories that the Arabs had used artificial insemination hundreds of years earlier for purposes of war, by inseminating, secretly, mares of their enemies with semen of inferior stallions, with the intention of weakening the strain, obviously have to be relegated to the realm of fiction; because in the light of the present knowledge of the difficulties involved, such an accomplishment could not have been possible.

The shifting of the large-scale use of artificial insemination to cattle and sheep did not take place simply because of the fading importance of horses; there were also reasons of a technical nature.

Shortly after World War I, Russian workers in animal breeding, attempting to apply the findings of Ivanoff in artificial insemination of horses to other classes of livestock, found that with these animals

progress was quicker and easier because the physiological mechanism of reproduction in cattle and sheep was more favorable than that in horses: sperm of bulls and rams are of a stronger constitution than stallion sperm, and in the females of these species the reproductive processes are less complicated than in mares.

In recent years considerable progress has been made in the treatment of stallion and jack semen, so that now artificial insemination is used with satisfactory results. Many difficulties are still encountered with the mare because of the complexity of her reproductive mechanism, even though these can be counteracted, to some extent, by artificial insemination itself and by hormonal therapy.

#### THE PHYSIOLOGY OF REPRODUCTION OF THE EQUINES

It is generally known that a mare stays in heat for many days. There was a time when it was believed that this extended period for mating should increase the fertility rate, on the assumption that by staying in heat for a long time, instead of only a few hours, a female had greater opportunity for becoming pregnant; for it was not recognized that there is only a short period in which the service or insemination must take place in order to result in fertilization and impregnation, namely, near the time of ovulation.

Contrary to the older opinion, ovulation is entirely independent of the act of copulation; it is brought on by other factors. At onset of heat the time of ovulation cannot be predicted, as the length of the interval between onset of heat and ovulation is highly irregular. Also, stallion sperm cannot live long in the mare; they survive only for a matter of hours, as a rule, between 24 and 48, and longer only in exceptional cases. Therefore, only the sperm of a service performed within this time limit can effectuate fertilization of the liberated egg; for those of a service given earlier will be dead by the time the egg becomes available for fertilization.

The reverse situation may happen, too. If a service is given too late in the heat period in relation to ovulation, the liberated egg will die before it can be reached by a spermatozoon; the unfertilized equine egg perishes within about five to eight hours after ovulation. Also, it takes the sperm five to eight hours to cover the distance from the site of deposition to the Fallopian tubes, where the egg is at this time (even though some sperm may be found there as

soon as 15 to 18 minutes after coitus). For this reason services given shortly after ovulation rarely, and those given later than two hours after ovulation hardly ever, result in pregnancies.

In the wild state, or under range conditions with the stallion running with a small herd of brood mares rarely exceeding twenty or thirty head, the stallion will serve a mare several times during her heat period; and under such a herd breeding system a pregnancy rate of 80 to 90 per cent is quite common. On the other hand, with the usual method of hand breeding as commonly practiced under farm conditions, frequently the mare is taken to the stallion or jack only once, usually as soon as she starts to show signs of heat. The pregnancy rate with this method is far below that with herd breeding, rarely more than 50 per cent and frequently much less. To imitate the herd breeding system with its high conception rate, but without overtaxing the breeding power of the stallion or jack, has become the chief function of artificial insemination in horse breeding.

This would require that the mares in heat be available for service during their entire heat periods. Under many breeding setups this is not practical, and it is not possible to give services at frequent intervals. For these reasons it is imperative for the successful operator to be able to deduct, from certain symptoms coincident with heat, when the best time to breed a mare is at hand. This holds true even though the modern breeder has now at his disposal means of regulating the natural course of the reproductive processes by the use of hormonal preparations. Through their proper application some of the obstacles can be reduced or eliminated, but hormonal therapy serves principally as an adjuvant to the natural mechanism. The methods of hormonal therapy suitable to horse breeding will be discussed later.

**The Breeding Season of Mares.** In horse breeder parlance the breeding season is that period in which the horse breeding operations are performed. It depends on economic and management factors, such as when it is most suitable to have the colts dropped, but it also is influenced by seasonal factors.

Mares under domesticated conditions and in moderate climates do not have a definite mating season; but the seasons of the year have a noticeable influence on the degree of reproductive performance, through an intermediary action of the nutritional regime and through

a direct effect of decreasing or increasing daylight and high or low temperatures

*Seasonal Influences on Mares and Jennets* Observations made at the Mississippi Experiment Station (Table XI) are presented to

TABLE XI. SEASONAL VARIATION IN THE BREEDING PERFORMANCE OF MARES, MISSISSIPPI EXPERIMENT STATION

Month	Length of heat cycles (days)						Duration of heat (days)												Percentage of heat periods resulting in pregnancies							
	15	16	19	22	25	28	Per cent of cases in each month																			
	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15	16	17	18	19	20	21	22	23	24	25		26	27	28	29	30	31	
Mar					31	68			23	23															34	14
April	3	13	14	19	22	25	3	1*	32	16	7	9	9	2	10											17
May	13	8	2*	2*	6	19	9	3*	24	14	11	2	2	1	2											45
June	19	28	21	14	10	17	17	33	33	5	2	1				1	1									41
July			50		25	25	4	31	54	7																50
Aug.			50	0				78		5	15															18
Sept.	34					68		39	39	60																73
Oct.					73	25		25	50	25																68
Nov				25	50	25			73	25																80
Dec.	60	140	116	85	222	294	27	191	21*	24*	35	13	31	5	49											

show that seasonal factors influence breeding functions, such as the heat period, the heat cycle, and the breeding efficiency. Similar observations have been reported by many other investigators from all over the world and were reviewed by the writer elsewhere.

Generally, a heat period of from three to eight days' duration is considered normal for a mare. Actually, most heat periods fall into the range of five to eight days, and only a small fraction of the heat periods lasts for less than three and four days.

The influence of seasonal factors is brought out by the fact that during the winter the breeding rhythm becomes extremely irregular, many mares stay in heat a long time, often for several weeks, and others fail to come in heat at regular intervals. Only a small percentage of mares become pregnant during seasons of irregular cyclic phenomena, and it is not advisable to continue breeding them the full time they are in heat in view of the low probability of conception. If they are still in heat after four services, they should be allowed to recuperate. As the season progresses, with better nutrition, prolonged illumination, and milder weather, the heat periods tend to shift into the range of the desirable short periods of three to six days, the heat cycles become shorter and more regular, and the settling percentages increase accordingly, with less work and

fewer breedings. It is interesting, and of practical importance to the horse breeder, that the return of a high state of reproductive capacity in the mare is preceded and advertised by the shedding of a rough winter coat of hair, according to investigations by English and Japanese workers.

It is equally important to know when to expect a mare to be back in heat after breeding so that it can be determined whether she became pregnant or not. It is an old practice to perform this check 18 days after the mare has been bred, regardless of the length of the heat period during which the service was given. Another method is to take the last day of the heat period, and to figure that 16 days after she ceased to show heat she will be back in heat if she has not become pregnant.

Neither of these two methods is entirely reliable because of the irregularity in length of heat cycles. From the presented data it can be seen that in the majority of cases the time between the first day of one heat period and the first day of the next will be 16 to 28 days, a wide range in itself. At seasons with unfavorable environmental conditions the length of the heat cycle in approximately 60 per cent of the cases is over 25 days; even under favorable conditions a substantial number, large enough for practical considerations, have a cycle longer than 25 days. The tendency is toward short cycles during the summer and long cycles in the fall and winter.

Also, the interval between two heat periods, when the mare is out of heat, is by no means constant at 16 days. It varies even during the favorable breeding months and is dependent upon the length of the preceding heat period—a short heat period is usually followed by a longer rest period and vice versa, but an individual may run every possible combination of long or short heat periods followed by short or long rest periods in spite of the seasonal tendencies.

One single checkup, made 18 days after a service has been performed, in numerous instances may fall either into the rest period preceding or into the one following the subsequent heat period. The same may happen when one depends solely on a rigid rest period of 16 days between heats. These unreliable checking methods are one reason why many mares do not have a foal every year. They are checked at the wrong time and are considered in foal if they are not receptive to the stallion, and they are not bred again when they come in heat later on.

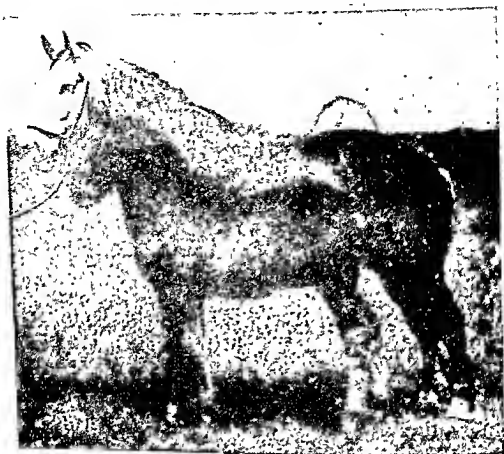


Figure 40. Percheron mare and colt owned by the Percheron Horse Association of America, Fair Oaks, Indiana. Present association rules provide that a colt produced by artificial insemination may be registered if the stallion and the mare were on the same farm at the time the mare was impregnated.

In order to avoid this, and in consideration of the irregularities to be anticipated with any one mare, it is good practice to begin checking 16 days after the first day of the heat period during which she was bred, and to continue to check every other day for at least another two weeks so that both types of mares, those with short and those with long heat cycles, can be caught if they return in heat.

Also, a mare may become pregnant and pass several heat cycles. Then, for some reason, pregnancy is interrupted, and the fetus is either discharged or reabsorbed. As soon as the uterus returns to the nonpregnant state, the mare resumes her heat cycles. For this reason mares presumably in foal should also be kept under observation throughout the entire breeding season.

The breeding habits of jennets do not differ fundamentally from those of mares, but some individuals may show still greater irregularities.

The length of the heat period varies in the same range as in mares, three to eight days, and is longer in unthrifty animals. In contrast with the mare, the jennet may have short heat periods of only one and two days' duration, not during summer, but in the fall; and consequently some jennets settle easier, with fewer services, at this season than in the spring and summer. The intervals between heat periods may become very long at any one season.

*Signs of the Best Time to Breed a Mare.* From the previous discussion one can recognize the difficulties confronting the horse breeder, especially when it is not possible to breed a mare several times throughout each heat period. The optimal time for insemination is several hours before ovulation, but the problem is to ascertain when it is going to happen.

The approach of ovulation can be ascertained by palpation of the ovary through the rectum, to determine the size and the condition of the follicle. This procedure requires great skill of the operator, and it is not infallible, since the size and consistency of the ovary and the follicle vary greatly from case to case.

More useful for the breeder are several other signs that appear during heat. They are brought on through the influence of hormones released from the ripening follicle, and they become intensified as maturation of the follicle progresses and liberation of the egg approaches.

The first of these signs is the behavior of the mare toward the stallion. Whereas at the beginning of heat she shows only a fair interest, her interest becomes more intensified the closer she is to ovulation.

The second indicator is the character of the vaginal discharge. At the start of heat it is stringy and glary. At the peak of heat, close to ovulation, it is present in profuse amounts and is of liquid consistency. It is either thrown off by the mare or is accumulated in the vagina. As the mare goes out of heat after ovulation, the mucus becomes more concentrated, sticky, grayish-milky in appearance, and finally a gummy mass that sticks to the walls of the vagina.

Some mares of sluggish nature show their reaction to the stallion in a very undecided manner, or do not exhibit any reaction. In such

cases one has to rely on a manual exploration of the vagina. It is obvious that for this kind of work close observance of hygienic conditions is essential.

In a mare not in heat the walls of the vagina stick together and are rough to the touch. With onset and progress of heat it becomes easy to introduce the hand, because the walls are now covered with slippery mucus, and they feel smooth and velvety. If these symptoms are present, it is safe to conclude that the mare is in heat.

Now one can proceed to obtain additional information concerning the stage of heat in relation to approach of ovulation. At early heat the cervix is firmly constricted, but with advancing heat the walls of the cervix become relaxed, soft, and flabby, first at the tip, and then throughout. Shortly before ovulation the tone of the cervix increases, it becomes sensitive to touch, resulting in more or less rhythmical relaxations and contractions, movements that apparently are connected with the process of receiving and transporting the semen during mating. At this stage, the cervix is centrally located and easily found, whereas at earlier heat stages it lies to one side, or on the floor of the vagina and is less accessible. If one palpates the ovary at this stage, one will find a large follicle that feels like a water-filled blister. Often the mature follicle can be ruptured by slight pressure.

This period, with all signs of approaching ovulation, may persist for several days, because sometimes the final ripening of the follicle before rupture is retarded, and ovulation is delayed. If no artificial means are applied to force ovulation, the breeder has to repeat services as long as signs for intensive heat and approaching ovulation are detectable, so that a constant supply of sperm will be available when the egg finally is liberated. If 48 hours after the last service the mare is still in heat, she has to be rebred.

The changes during heat, as described for the mare, are also found in the jennet. Usually, but not always, intensity of heat in a jennet can be recognized by her peculiar chewing movements. She also will ride other jennets or stand to be mounted, acting in this respect more like a cow than a mare. However, by no means are all jennets easily detected when in heat. Many will show these symptoms without the presence of a jack, others have to be checked very carefully with a teaser. In jennets that fail to demonstrate the outward signs of heat, the internal changes have to be relied upon for determina-



tion of presence and stage of heat. They are identical with those described for mares.

**Breeding at Foal Heat.** When considering the best time to breed a mare, the question of breeding at foal heat always comes up, because for a long time breeding on the ninth day after foaling was considered the safest way to produce pregnancy. Investigations in recent years have shown that foal heat may start as early as two days and as late as eleven days after foaling, and even later. Foal heat may last just as long as any other heat period. If the "ninth-day service" is followed by cessation of heat 48 hours later, the chances for conception are good. If the service is given on the first day of a heat period of, say, five days' duration, the chances are less. Hence breeding on the ninth day after foaling is by no means a guarantee for conception.

Furthermore, it was observed that in many mares the uterus has not "cleared up" by foal heat, and is especially apt to become infected. Many progressive horse breeders have abolished breeding at foal heat, and start breeding during the next heat period, which in most cases will set in 26 to 30 days after foaling.

#### HORMONAL THERAPY

The role of hormones in reproduction and hormonal therapy is discussed in a separate chapter. Here, only those methods of hormonal therapy will be discussed that are applicable to the correction of physiological inadequacies in the mare, namely subnormal ovarian function and late ovulation in the estrous period.

Subnormal ovarian function appears in three forms: as deep anestrus, as shallow anestrus, and as incomplete manifestation of heat, usually referred to as "silent heat."

Deep anestrus with cessation of all ovarian activity is fairly rare in the healthy mare, but it may set in under extremely adverse climatic and nutritional conditions and is a natural occurrence with advancing age. The ovaries become small and shrunken, are of an unelastic consistency due to a preponderance of fibrous tissue, and contain no palpable follicles. Hormonal therapy of all kinds has proved to be useless, because such ovaries, due to a low blood supply, seem to be incapable of responding to a hormonal stimulation. If caused by environmental factors, spontaneous recuperation

may set in with improved nutritional and seasonal factors, without any further medication

In mares in shallow anestrus, and also in those exhibiting "silent heat" behavior, the ovaries do function, but at a subnormal level. They grow cyclic follicles, but these apparently fail to produce sufficient amounts of estrogen to make the mare show heat. Palpation of the ovaries and the vagina would reveal that cyclic changes take place. The ovaries are of a spongy consistency and contain palpable follicles of about 1 to 2 cm in diameter, and the vaginal walls are covered with mucus.

Some mares stay persistently in shallow anestrus even during the true breeding season, possibly due to a genetically inadequate hormonal apparatus that causes this condition of subfertility, just on the border of sterility, but a state of shallow anestrus is a seasonal appearance in most mares as a transitional stage before the onset of the season of highest reproductive capacity.

Shallow anestrus and silent heat can be corrected by hormonal therapy. It has been reported that follicle stimulating hormone (PMS) injected subcutaneously in doses of 1,000 international units stimulates the underactive follicle to grow to maturity, and to secrete sufficient amounts of estrogen to make the mare come in heat, and to initiate normal cyclic behavior. It also has been reported that both these defects can be corrected by subcutaneous administration of rapid acting estrogen preparations in quantities small enough to stimulate the pituitary into increased activity, 10 to 15 mg of dienestrol or stilbestrol in a suitable vehicle will induce stimulation of an undeveloped follicle toward maturation and ovulation, accompanied by manifested heat. It takes about four to eight days for this development, so that service can be performed approximately one week after treatment. Slow-acting estrogen preparations or too large doses have the undesired effect of still further suppressing the already hypoactive pituitary.

Late ovulation, typical even in normal mares, also can be corrected with hormonal therapy. Intravenous injection of the luteinizing gonadotrophic principle from human pregnancy urine in amounts of 1,000 to 2,000 international units, administered after the mare has come in heat, induces ovulation in 24 to 48 hours. Identical results are also obtained with the subcutaneous injection of 5 to 10 mg of dienestrol, also applied after the mare has come in heat. The

administered estrogen presumably stimulates the pituitary to release the luteinizing principle at an earlier stage, thereby forcing ovulation sooner than it would occur without stimulation. Insemination or natural service should be performed 12 to 18 hours after treatment.

#### SEMEN PRODUCTION OF STALLIONS AND JACKS

Semen production of stallions and jacks differs greatly from that of bulls and rams; it is in many respects similar to that of the boar. Stallions and jacks discharge large volumes of semen, an ejaculate ranging from 25 to 150 ml., although some individuals give as much as 400 ml., and with others as little as 15 to 20 ml. may constitute a complete ejaculate. Stallions of the warm-blooded breeds in general give the smaller ejaculates, but these are of a higher sperm concentration than the voluminous ejaculates from draft stallions and jacks. After a sexual rest period the first ejaculates tend to be larger; and, inversely, in too frequently used animals the later ejaculates become smaller. The drop in semen quality with too frequent services is more rapid in stallions and jacks than in bulls and rams. Only highly fertile animals can give two services daily, and then only for a short period; but a regime with one service every other day throughout the breeding season apparently provides a sufficient time interval for recovery. Experiments, and practical experience as well, have shown that feeding exerts a significant influence on the semen-producing performance. Animals either in a too high condition or in an unthrifty one produce semen of low sperm content and low viability. Green grass and oats are essential for high-quality semen production.

If studs are not used for several weeks, the first ejaculates may contain a large proportion of dead and stale sperm, and this semen should not be used for breeding.

The semen of stallions and jacks is much thinner than that of bulls. The range of sperm concentration is rather wide, between 30 and 800 million sperm per ml., depending on the sperm-producing capacity of each individual, and also on the feeding and service regime. Semen with a sperm concentration of less than 100,000 sperm per ml. is not suited for artificial insemination.

Stallion and jack semen differs from that of other species also in

regard to the significance of the proportion of morphologically abnormal sperm forms: An abnormality count of 300 per 1,000 sperm is encountered even in semen of highly fertile stallions and jacks, a proportion that in bull and ram semen would indicate impaired fertility. In overconditioned animals that do not get enough exercise, and during extremely hot weather, the abnormality count increases, especially the forms with head abnormalities, including very small heads; and a large proportion of sperm will be dead at collection or will die within a few hours.

Stallion sperm and jack sperm have a low viability. Even in modern diluents they survive only for a few days and retain their fertilizing capacity for less than two days.

*The Physiology of Semen.* The sperm cells of the stallion and jack, like those of other animals, utilize as sources of energy the sugars present in the semen. However, the sugar content in stallion and jack semen is relatively low and quickly exhausted, so that the sperm perish from starvation. Also, natural anabiosis due to acidification by lactic acid formation does not occur because of the alkalinity of stallion semen, which is caused by the high proportion of accessory seminal fluid. The seminal fluid from the accessory sex glands is also high in salt concentration, and this stimulates motility of the sperm cells, thus inducing their death by exhaustion. It also has an adverse effect on the capsule of the sperm head by causing its destruction. This capsule of stallion sperm is reported to be structurally weaker than that of bull and ram sperm.

With natural service and with artificial insemination alike, semen cannot be deposited into the sperm life-preserving area of the cervix, because in the mare the cervix is not anatomically equipped to serve as a holding space for the semen. It is deposited instead into the uterus. The environment in the uterus, however, is not favorable for prolongation of the life span of sperm. Therefore, prolongation of sperm survival both *in vitro* before insemination and in the uterus after breeding has to be achieved through treatment of the semen with proper diluents that counteract the harmful effects of the constituents of seminal fluid and of the uterine environment. Such diluters are described in a later section.

Experiments have shown that stallion and jack sperm survive best when the semen is diluted in a ratio of 1 part semen to 3 to 5 parts of diluter. Above this ratio the time of survival decreases with in-



Figure 41. Grade Shetland ponies raised by Bells' Pony Farm, Bellmawr, New Jersey. The two ponies under saddle are the small type, under 42 inches high.

creasing dilution rates, until with a dilution of 1 to 10 the survival period is only slightly longer than that of undiluted semen. Semen of high density, as produced by highly fertile stallions, is less subject to the unfavorable effects of high dilution rates than thin semen from poor producers.

Another factor regulating the degree of dilution is the required sperm number per insemination dose. Experimental breeding results indicate that each dose should contain not less than 1 to 2 billion sperm.

The most important consideration for dilution extent is the size in volume of each dose. It is now well established that in equines large volumes are more advantageous than the small volumes that were used in earlier days. For small and open mares and jennets, doses of 10 to 20 ml. are adequate; but for large mares and for those in which the uterus is still extended through a preceding pregnancy, the dose has to be at least 30 to 40 ml. Still larger doses have to be given if the semen is originally of low concentration. Even though such semen would provide the necessary volume without further being diluted, it still should be diluted at least at a 1:1 ratio in order to introduce the protective ingredients in the diluter. To

compensate for the now reduced sperm concentration, the injection of doses amounting to over 100 ml occasionally may become necessary, but these can be handled with the now available instruments

The reason for the need of these large insemination volumes in equines is not well understood, but it may have its explanation in the anatomical structure of the mare's uterus and in the site of semen deposition. With artificial insemination of cows and sheep the fact that one quarter to one half of the insemination dose is deposited in the cervix improves the chances for sperm preservation, and thus only fractions of the ejaculate are needed. This is not true in the mare, and here the large volumes of the insemination dose apparently are needed for more adequate transportation of the sperm mass. When stallion semen that has been stored is used for insemination, it is well to remember that the survival period of the sperm in the uterus decreases proportionally to the length of the preceding storage period. Therefore stored semen has to be given at frequent intervals, not more than a day apart, so that a new supply of fresh, even though short lived sperm is available, and close observation for approaching ovulation becomes mandatory in order to time insemination to ovulation. For the time being, in view of the unsatisfactory results with storage of stallion semen, the use of stored semen is not well adapted to routine breeding practices.

#### SEMEN COLLECTION

In earlier days, a widely used method of obtaining semen was that of collecting the last parts of the ejaculate as the stallion dismounted. This method, although simple, is inadequate, not only because of the inferior quality of this semen fraction but also because it frequently becomes contaminated with dust and dirt and because sufficient amounts are not collectable. Another method was to let the stallion or jack serve a mare and to collect the semen out of the mare. The disadvantages here are that the semen gets mixed with the contents of the reproductive tract of the mare and becomes unsuited for storage. Furthermore, if the mare harbors any kind of infectious bacteria, they will be carried with the semen to other mares. For these reasons these methods are no longer recommended.

! Quite satisfactory results can be obtained with the so-called breeder's bag because the semen collected in this manner is free

from the contaminations of the mare. This bag is made of rubber and is applied to the penis of the stallion before he mounts. It is difficult to apply on some stallions and jacks; it also often slips off as he dismounts. If the outside of the bag gets covered with the mucus from the mare, the semen may become contaminated with disease-carrying germs from the mare as it is poured out of the bag. Unsatisfactory for the same reasons were the methods in which a bag-like contraption was placed in the vagina, serving as a receptacle for the ejaculate; it also was extremely difficult to adjust this kind of semen collector in the vagina.

Finally, a method was worked out whereby the semen could be collected in an artificial vagina. For the work with bulls and rams, the first models of this type were so satisfactory that hardly any improvements had to be introduced, but considerable difficulties were encountered in developing a suitable model for stallions and jacks.

*The Artificial Vagina.* The original artificial vagina for stallions, constructed by Russian and British workers, consisted of a metal tube, about 15 to 20 cm. in diameter, ending in a cone, to which was attached the semen receptacle. The water jacket was formed by a rubber lining as in the vagina for bulls. This outfit was heavy and complicated to handle. In an attempt to develop a more efficient instrument, two models were constructed independently by American workers, the so-called Missouri-USDA model by McKenzie, and the Mississippi model by the writer.

As these pieces of equipment are not commercially available, a description of their structural features is given here so that they can be assembled by prospective users.

The Missouri-USDA model was described by Lambert and McKenzie (1940):

"The Missouri-USDA model of the artificial vagina for horses consists simply of a rubber tube about 18 inches long and 17 inches in flat diameter, with a rubber ring placed in the open end and the other end narrowed down to stretch over a bottle. In order to develop the proper pressure, a second rubber tube of the same size is drawn over the first, the two tubes are then vulcanized together at each end, and an ordinary tire valve is placed in the outer tube so that air may be pumped between the two tubes. A leather casing surrounds the rubber tubing, giving it rigidity, and a handle grip is attached to the casing. An essential feature of this model is the 3-inch rubber band

placed around the inner tube near the open end. This simulates the sphincter muscle of the mare and aids materially in making collections from stallions."

The Mississippi model, shown in Figure 42, was built on the principle that stimulation for ejaculation in the stallion is brought on, not through the sphincter, as stated by McKenzie, and as it is in the bull and ram, but rather by the pressure of the anterior vaginal wall against the glans penis. This effect cannot be obtained in the open ended models, and therefore in this model the forward end is closed off by a clamp that is removable in order to facilitate cleaning.

An improved version of this model is made of one piece of rubber tubing 36 to 48 inches long and 7 to 8 inches in flat diameter. To provide for the water jacket the tubing is turned inside out over a stiff rubber ring that forms the entrance end. This ring is suitably made from an 18-inch long piece of rubber tube with ends joined together by an inserted piece of wood. After the ring is manipulated into the proper position, it is fixed in place by cementing together the inner walls of the tube by two or three patches.

A water inlet is provided by vulcanizing into the outer wall a stub of rubber hose that can be closed by a rubber stopper.

For the outflow of the ejaculate, a concentric hole of approximately  $1\frac{1}{2}$  inches in diameter is cut into the outer and inner layer, and the edges around the hole are cemented together. At the same time a stub of rubber hose is fitted into this hole to serve as the connector to the semen receptacle. Next, the edges of the outer and inner layer at the other end are cemented together so that now the entire structure is a two walled tube open at both ends.

Two loops of rubber material are cemented to the outer wall to serve as handles, as shown in the picture. The clamp for closing one end is simply made from two strips of hard wood with two thumb-screws.

When the vagina is made ready for use, the clamp is put in place, and the water jacket space is filled with hot water until the pressure in the vagina is adjusted according to the size of the studs and their individual preference. The temperature has to be regulated so that it is between  $41^{\circ}$  and  $45^{\circ}$  C ( $105^{\circ}$  to  $112^{\circ}$  F) in the interior of the vagina. Then the entire inside wall is covered with a nonspermicidal lubricant.



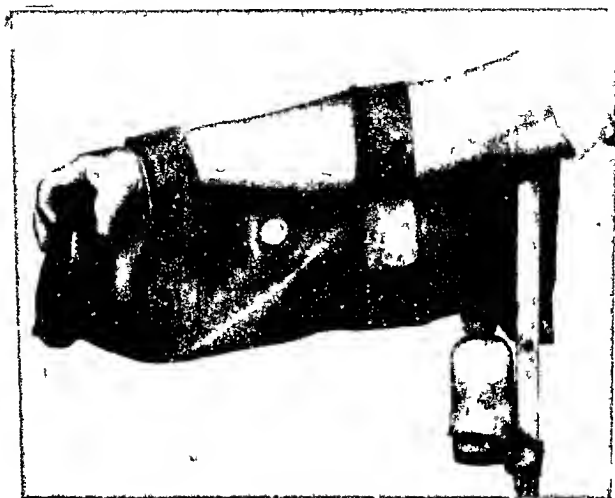


Figure 42 Artificial vagina for stallions Mississippi Model U.S.A.

A mare in heat is used at the time of collection, and as a precautionary step she should be restrained by hobbles and her tail wrapped by a bandage. Stallions and jacks can also be trained to work on a dummy. Electroejaculation to our knowledge has not been tried with stallions.

As the stallion mounts, the penis is guided by hand into the vagina, but the vagina must be held firmly against the flank of the mare and not pushed towards the stallion, so that the stallion has to make the forward thrust that elicits ejaculation as soon as the glans hits the anterior wall. Manual pressure is also helpful.

Unlike the bull, which gives off the ejaculate with one sudden thrust, the stallion and jack ejaculate for about 10 seconds, and the semen is expelled in several fractions. The first fraction is of watery consistency and does not contain live spermatozoa. During the subsequent main phase the ejaculate comes from the testes and contains spermatozoa in high concentration. This fraction has a milky

appearance due to the presence of spermatozoa. During the final phase, a glary, viscous material is expelled, resembling egg white, that comes from the secondary sex glands, the seminal vesicles, and Cowper's glands, which contribute the bulk of the seminal liquid but do not produce spermatozoa.

The discharge from these glands may make up half of the volume of the total ejaculate of draft stallions, in semen of jacks and saddle stallions it is present in smaller amounts. In the earlier days of the practice of artificial insemination this portion was caught in a container and used for artificial insemination, because this fraction was easy to obtain, being partially discharged as the stallion dismounts. The results of this system were frequently unsatisfactory, for the sperm concentration in this portion is only about one tenth of that in the sperm-carrying portion of the ejaculate, and therefore not enough sperm were introduced into the mare.

#### HANDLING AND TREATMENT OF SEMEN

Since the sperm cells are very sensitive organisms, cleanliness is absolutely essential. Exposure to air is not detrimental, but it increases the probability of contamination with bacteria and dust. Therefore semen should be kept in closed containers. Ordinary drug-store medicine bottles, with graduation marks to make the addition of diluter more accurate, are very suitable for this.

Diluter is added to the semen as soon as it is collected, regardless of whether it is to be used for immediate inseminations or for storage and shipping. It must be warmed to approximately body temperature before it is added. It is mixed uniformly with the semen by rotating movements, avoiding vigorous shaking which is harmful to sperm.

With semen containing a large portion of the glary, viscous material, some difficulty may be encountered in obtaining an even mixing. Since this viscous fraction interferes with the storing qualities of semen, it is best to separate it from the sperm-containing fraction immediately after collection. This is done by tilting the bottle until the mass of the glary material can be pushed out with a glass rod.

After dilution the bottle with the diluted semen is placed in water of  $20^{\circ}$  to  $27^{\circ}$  C ( $70^{\circ}$  to  $80^{\circ}$  F) in this condition it is kept ready for insemination within the next few hours.

If it is necessary to use part of the same ejaculate on the following

days, it is essential to divide the ejaculate, after it is diluted, into doses of proper size in small containers so that only the dose that is to be used has to be reheated, thus avoiding resuscitation of sperm in those portions that will be stored longer.

When preparing semen for storage, it is essential to cool it as soon after collection as possible in order to bring on quickly that state of reduced motility of sperm cells which is necessary to preserve their energy.

With egg yolk diluter the cooling process can be rapid. Placing the bottle into a refrigerator or into a thermos jug containing ice water with a temperature of 5° to 10° C. (40° to 50° F.) is the most effective measure.

#### PRESCRIPTIONS FOR DILUTERS

In recent years there has not been much activity in the search for new improved diluters for stallion semen.

Table XII, based upon work at the Mississippi Experiment Station, gives the composition of several diluters and their efficiency.

TABLE XII. COMPOSITION OF DILUTERS AND THEIR EFFECT ON THE LONGEVITY OF SPERM OF JACKS AND STALLIONS

Formula and items compared	Diluter *					
	TGL-2	TGL-5	TGL + gelatin	TGL + egg yolk	TGL + egg yolk + gelatin	Egg yolk + phos.
Formula of diluter, in gm. per 100 c.c. water:						
Glucose	5.76	6.48	5.76	5.76	5.76	(2.0 gm.
K-Na tartrate	.67	.33	.67	.67	.67	Na <sub>2</sub> HPO <sub>4</sub>
Tartaric acid		.01				+ 0.2 gm.
Tannin		.002				KH <sub>2</sub> PO <sub>4</sub> )
Peptone	.2	.8				
Egg yolk (c.c.)				30	30	100
Gelatin, as capsules			1.8		1.8	
Remarks	Suit-able for immediate use	Suit-able for 24 hrs. storage	Not suit-able for storage	Suit-able for storage	Very good for storage	

\* For a discussion of the TGL diluters, see Milovanov, 1936.

The egg yolk-phosphate diluter as presented in the table has the same composition as the one used for cattle. In the experience of the writer, it has not given quite as good results as the tartrate diluters, but Missouri workers stated that addition of 10 gm glucose to the egg yolk phosphate increased its efficiency as stallion semen diluter.

Other investigators found later that for stallion sperm adequate amounts of egg yolk and glucose are more important than the buffers in a diluter, for a simple diluter made up of equal parts of egg yolk and 5 per cent glucose solution gave better sperm survival than the buffered egg yolk diluters.

French workers reported recently that superior sperm survival is obtained when the egg yolk in the egg yolk tartrate diluters is replaced by boiled, filtered milk, and survival for at least four days resulted if condensed milk was used in place of natural milk. This diluter consisted of equal parts of commercial condensed milk with 60 per cent water content, and Milovanov's diluter, containing 6.85 per cent glucose, 0.15 per cent K Na tartrate, and 0.008 per cent tartaric acid, to which were added 0.6 per cent sulfanilamide.

Deep freezing of stallion semen has not been studied to a great extent, and so far it seems that the method so highly effective for bull semen is not applicable to stallion semen.

The usefulness of chemotherapeutics and antibiotics for stallion semen diluters has not been investigated to the same extent as for bull semen diluter. It appears that about 100 to 200 units of the antibiotics per ml diluter and 0.3 to 0.6 per cent sulfanilamide exert an antibacterial effect on stallion semen without damaging the sperm.

#### INSEMINATION OF THE MARE AND JENNET

Before the mare or jennet is inseminated she must be restrained by hobbles or put in a breeding chute or she can be backed against the teasing pole.

The tail is wrapped in a towel and held to one side. The region around the vulva is cleaned and disinfected by washing with disinfectant solution. Next a coat of lubricant is applied to the vulvar region.

For the following operations the operator should wear rubber gloves which are washed off with disinfectant between each successive manipulation. This precaution has to be observed rigidly,

because by neglecting it a rapid spread of infectious diseases of the sex organs of the females may result and nullify the benefits of artificial insemination.

Before insemination it is advisable to insert the hand into the vagina and to scoop out the accumulated mucus, urine, and so on. This is especially important in aged mares in which the position of the pelvis has changed so that the floor of the vagina is lower than the vulva. If the accumulated material is not removed, it may flow into the cervix during insemination and damage the deposited semen. Care should be taken when introducing the hand that no air flows into the vagina, since stable air is always filled with bacteria and dust.

The next step is insemination. Two methods are commonly used. In one the diluted semen is poured into a one-half-ounce gelatin capsule holding 10 ml. of semen, and the capsule is quickly introduced by hand into the cervix and pushed with the forefinger well into the uterus. For large and nursing mares several capsules are given successively, or else the entire dose is given in one-ounce capsules.

With the other method the semen is poured into a bottle holding about 40 to 50 ml. (1 to 1.5 oz.), which is equipped with a rubber stopper with two glass tubes, a long one reaching to the bottom of the bottle and a short one reaching just below the stopper. To the long tube is attached a catheter rubber hose of approximately 30 inches, and to the short tube a pressure rubber bulb or simply a short rubber hose. The catheter hose is introduced by hand into the cervix and into the uterus for about ten inches, the cervix is clamped off with the fingers, and the semen is now pumped into the uterus by the rubber bulb.

The second method is useful for insemination of jennets, because they have a longer and more tortuous cervix than mares, which makes it difficult to introduce a capsule, and also for maiden mares when the cervix is too constricted to admit a capsule freely. Its chief advantage comes when large nursing mares have to be bred, and when semen of low sperm density has to be used, both cases requiring large doses of 60 to 120 ml. (2 to 4 oz.), which would have to be given in several capsules.

Deep deposition of the semen is essential in a mare with a loose cervix with insufficient tone to close firmly after service.

Between inseminations of each mare the catheter tube is thor-

oroughly cleaned and disinfected to eliminate the danger of spreading disease. It is safer to have a number of interchangeable catheters and to use a fresh one for each mare.

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# Poultry

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Artificial insemination of poultry has become established as a valuable technique in both industry and research. Although the commercial chicken breeding flock is seldom artificially inseminated, specialized breeders have found many ways to use the technique. The turkey industry has utilized artificial insemination to a much greater degree than any other in the poultry field. Much of the progress in breeding heavy and broad breasted turkeys has come about through extensive use of artificial insemination. As a matter of fact, some of the larger and meatier strains can barely reproduce themselves naturally, but over 80 per cent of their eggs will be fertile following artificial insemination. Very little artificial breeding of waterfowl has been practiced to date, but it would not be surprising to find considerable acceptance in the future. Artificial insemination has proved to be a particularly valuable tool in the field of poultry research.

According to Griffin (1938), Ivanoff artificially inseminated birds as early as 1902 by killing the male and then drawing off semen from the terminal bulbs of the seminal ducts, but it is apparent that this method would have only limited application. Payne (1914) allowed chickens to mate naturally and then quickly removed the semen from the cloaca with a small spoon. It was found possible to inseminate other hens with this semen. Amantea (1922) collected semen from the male domestic fowl by intercepting the semen just prior to the



consummation of a mating. The methods of Payne and Amantea require the presence of an attendant at the time of natural mating, and in addition the technique of the latter requires an unusual amount of alertness and dexterity. Other techniques were developed in which semen collectors were fastened either to the body of the male or to that of the female, thereby collecting the semen automatically when the birds attempted to mate naturally. For example, Ishikawa (1930) collected semen from the domestic fowl by fastening an artificial cloaca on the hen. Tinjakov (1933) described an artificial semen collector which is fastened around the cloaca of the male. Successful semen collections have been made with electrical stimulation from both chickens and geese by Serebrovsky and Sôkolovskaya (1934) and from ducks by Watanabe (1957).

Quinn and Burrows (1936) and Burrows and Quinn (1937) were the first to describe a practical method of semen collection. Their manual massage technique is the basis, with slight modifications, for all modern techniques involving the collection of semen from chickens, turkeys, or waterfowl. This method can be used at any time convenient to the operator, provided the male bird is properly handled.

In poultry the term "fertility" means only that embryonic development has been initiated. "Hatchability" is the term used when describing the relative success of chicks in emerging from the shell. For example, a fertility of 90 per cent and a hatchability of fertile eggs of 80 per cent would result in a 72 per cent hatch of all eggs set.

#### THE CHICKEN

*Male and Female Reproductive Systems.* The anatomy of the chicken male reproductive system is remarkably different from that of domesticated mammals. The testes are located within the body cavity—which would cause sterility in most mammalian species. There is a duct system consisting of paired epididymides and vasa deferentia. Accessory glands, such as seminal vesicles, Cowper's gland, or a prostate gland, are not to be found. The vas deferens does increase slightly in diameter just prior to entering the cloaca, and some semen is stored in this bulbous region. The chicken has no functional counterpart of the mammalian penis, although a rudimentary penis or copulatory organ is present in the cloaca. The

paired vasa deferentia terminate instead in double teat-like structures known as papillae. These are clearly in evidence following sexual stimulation, and it is from them that the semen is milked.

The reproductive system of the hen has evolved in a special manner so as to produce the highly complicated egg. The upper region of the oviduct receives the yolk following ovulation from the ovary. Much of the oviduct is concerned with the secretion of materials to form albumen and shell membranes. The uterus or shell-secreting pouch is located about one fourth of the way up the oviduct from the cloaca. The short portion of the oviduct connecting the cloaca with the uterus is the vagina, this is the part seen when the oviduct is everted for insemination.

**Equipment for Artificial Insemination.** The minimum artificial insemination equipment needed for the chicken is shown in Figure 43. A collection cup is needed to gather in the semen as emitted. One desirable type is a small glass or plastic funnel with paraffin sealing off the narrow outlet tube. The outlet tube may then be stuck into



Figure 43 Simplest artificial insemination equipment for poultry, including the container of 1 per cent salt solution, the inseminating syringe, small storage tube and the collection funnel.

a cork or rubber stopper. The distance from the stopper to the funnel lip may be varied to suit the size of the hand of the operator. A one-cubic-centimeter tuberculin syringe, graduated in hundredths, is an ideal vehicle for transferring measured dosages of semen into the oviduct of the hen. A 1 per cent solution of table salt should be available to rinse the equipment between samples when pedigreed males are being handled. If semen is being collected from a group of males, and then mixed prior to insemination, some type of storage tube should be available. If one attempts to collect a number of samples of semen from different males in only one collection cup, there is the danger of contamination of the combined samples by one contrary male.

**Management of the Cock.** The cock must be segregated from hens, and preferably from fellow cocks. It is usually impossible to collect semen from a cock running with hens. Best results are obtained when the males are caged individually; small wire cages, 20 by 20 inches, with either wire or wooden floors are satisfactory. Males should be isolated from females at least 24 hours before attempting to secure semen from them. It is helpful to handle and ejaculate the male several times before making a serious attempt to obtain semen, although some males respond well on the first attempt. Thoroughly trained males may have to be handled carefully and quickly to avoid loss of semen through premature ejaculation. Neither semen yield nor semen quality is adversely affected by collections as often as three times a week for indefinite periods. If there is a problem of contamination by watery or loose droppings, it usually pays to increase the feeding of whole cereal grains and cut down on mash or pellets.

**Collecting Semen from the Cock.** The simplest way to collect semen by the massage method requires two operators. One holds the bird while the second concentrates on stimulating the flow of semen and catching it in a funnel or cup. As will be described later, one man may do the entire job, but considerable skill is required and there are some definite disadvantages. The one holding the bird should grasp the thighs and at the same time hold some of the wing feathers to prevent flapping. The bird should be held in a horizontal position at a height convenient to the operator who is attempting to secure the semen. The bird may be held either under the arm or against the body.

To collect semen the operator should place the palm of his left hand against the fleshy tail and at the same time push the tail feathers up and out of the way. The thumb and index finger of the left hand should be in position on either side of the cloaca and close to it. The left hand now is in the appropriate position to squeeze out semen at the appropriate time. The right hand holds the collection funnel. Since both the thumb and the index finger are needed for the massaging process, the cork or holding part of the collection funnel must be held by the remaining fingers. Operators differ in the number of fingers required to hold the cork, but in general the more fingers available for massage the better the results. The sides of the soft part of the abdomen are massaged at a level beneath the pelvic bones. Massaging should be rapid and continuous until the male responds by protruding the papillae from the cloaca. Once the papillae are fully protruded, the previously positioned thumb and index finger of the left hand can milk or squeeze out the semen (see Figure 44).



Figure 44 Collecting semen from the cockerel. Note position of thumb and index finger of left hand

Since the semen is stored in the bulbous vasa deferentia in back of the papillae, it is important to start the squeezing from a position back of the fully protruded papillae; the most common mistake of beginners is to squeeze too soon or before protrusion is complete. Several squeezing motions should be made in order to secure maximum yields.

One-man methods of semen collection involve either the use of a mechanical holding device or the ability of one person to hold, stimulate, and collect the semen by himself. Homemade stands with adjustable straps may be used to secure the male, but they are slow to work with, and frequently either overly excite untrained males or stimulate semen emissions before they are wanted from trained males. A useful device has been described by Gabriel (1957) which should eliminate these disadvantages. Her method involves the use of a metal cone shaped like the conventional one used in killing chickens. The male, with head down, is put into the cone so that his legs and abdomen protrude from the rear. The exact size of such a holding cone would depend on the size of the bird. One suitable for average-sized males is 11 inches long with a 6-inch diameter at the wide end and a 5-inch diameter at the narrow end.

The simplest one-man technique was described by Mueller (1949). The operator sits down and holds the male in his lap with the head of the male facing to his left. The bird is held in place by securing his legs between the knees of the operator. Both hands are then free to proceed with the conventional massaging. Another method has been described by Wheeler (1948) in which the male is held between the thighs of the operator. By standing in a knock-kneed position he may hold the male with a minimum of pressure. Initial sexual excitation is produced by stroking down the back several times with particular emphasis in the region of the fleshy tail. As soon as the papillae protrude, the thumb and index finger of the left hand should be placed in position on opposite sides of the cloaca. Then, quickly picking up a collection funnel with the right hand, the operator squeezes semen into it with the milking motion of the thumb and index finger of the left hand.

Since the urinary and digestive tracts also empty into the cloaca, it sometimes happens that semen samples become contaminated with either fecal material or white chalky urates. It has been our experience that a small amount of urates does not adversely affect fertilizing capacity of the semen sample, if used shortly thereafter. Fecal con-

tamination does reduce fertilizing capacity, and unless the fees are removed promptly it is best to discard the sample. Occasionally small amounts of blood become mixed with the sample because of injury to the papillae. For the most part such contamination is not serious, although it may not be desirable to include these collections in a pooled semen sample. Males which pass blood should be rested for a few days and handled carefully thereafter.

*Inseminating the Chicken Hen* The most common method of inseminating the hen is the one described by Burrows and Quinn (1937). It requires two people with the first operator holding the female firmly by the lower thighs with his left hand and at the same time holding the hen against his body. The right hand is used to evert the oviduct. To do this the thumb is placed above the cloaca and the fingers and palm on the abdomen below the cloaca. Eversion is accomplished by exerting pressure with the right hand. Since there is but one functional oviduct—the left—vaginal protrusion will be noted in the left side of the cloaca. The opening in the right side of the cloaca is that of the intestine. Eversion is not difficult to bring about in laying hens unless they are overly fat, in which case additional pressure may be required. Eversion of the vagina in non layers is usually not possible. As soon as the oviduct is everted, the second operator inserts the tuberculin syringe to a depth of about one inch (see Figure 45). Just before injecting the semen, the abdominal pressure is released to avoid squeezing the semen back out of the oviduct. Once the oviduct has returned to its normal position the bird may be released.

Mueller (1949) has described a one man technique for inseminating females. The hen is held securely in the left hand with its right leg between the index and second fingers and its left leg between the third and fourth fingers. Unless there is excessive struggling the middle fingers may also be used to apply pressure to the lower abdomen. Next the right hand is used in everting the oviduct, and once it is everted enough pressure should be applied with the middle fingers of the right hand to keep or hold the eversion. The right hand is now free to be used for injecting the semen. Mueller recommends a graduated medicine dropper although the one cubic centimeter syringe can also be used by operating the plunger with nudges by the thumb.

Several modifications of the insemination technique have been



Figure 45. Inseminating the hen by the conventional two-man method.

developed for inseminating hens in caged layers without removing them from the cage. According to Moultrie (1956) two men can inseminate approximately 40 more caged hens per hour with his method than with the conventional technique. With his right hand the operator grabs the hen by the hocks and pulls the feet and shanks through the cage door, leaving the body of the bird resting on the floor of the cage. The legs must be held firmly together so as to exert some pressure on the anterior abdomen. The left hand is then used in everting the oviduct, and a second operator injects the semen (see Figure 46). A similar technique requiring but one operator has been described by Gabriel (1957). With this method the thighs of the bird are held tightly from underneath by the fingers and palm of the left hand. Holding the thighs in this position results in a partial crossing of the shanks and exerts an upward pressure on the oviduct. The thumb of the left hand is used to apply lateral pressure on the side of the abdomen. The combination effect forces out the oviduct, and the semen is then deposited by means of



Figure 46 Inseminating hens maintained in laying cages according to the method described by Moultrie (1956) (Courtesy Alabama Agricultural Experiment Station)

the right hand. Hens may be inseminated at the rate of 200 per hour with this method.

Direct deposition of semen into the uterus rather than the vagina has been shown recently to increase fertility under certain conditions (Allen and Bobr 1955, Allen and Grigg 1957). This technique should be of value for inseminating low fertility lines and for use of semen of questionable viability for example, after freezing or after a holding period. It is necessary to use a 6 to 10 cm glass cannula as an extension for the regular syringe tip. The cannula must be guided through the coils of the vagina and into the uterus, where the semen is released. Previously, it is necessary to insert the forefinger into the vagina and probe to find the junction of the uterus



and vagina. Then the cannula is inserted and guided along the finger into the uterus. With experience it may be possible to reach the uterus without probing with a finger.

A completely different approach has been used by Van Drimmelen (1945). With this method the semen is deposited through the side of the body directly into the body cavity in the region of the ovary and upper oviduct. Considerable skill is required if serious damage is to be avoided. This technique might have considerable value in research studies of hens which remain infertile after insemination by conventional methods.

Moore and Byerly (1942) found that the presence of a hard-shelled egg in the uterus greatly reduced the chances of a fertile insemination. Accordingly, it is desirable to inseminate during that portion of the day when the fewest number of birds will have hard-shelled eggs in their uteri. This will usually be between 1 and 5 P.M., varying in part on the stage of production and on the artificial lighting program being used. An egg in the uterus can be detected with ease by means of palpation. This is accomplished by inserting a finger into the intestinal tract and feeling for an egg. By this method it is possible to distinguish between eggs with no shell, slight shell formation, and hard shell. The authors have found it safe to inseminate up to the point where the forming egg shell is no longer crunchy. For maximum fertility, all hens should be palpated prior to insemination.

*Semen Yields, Dosages, and Intervals Between Inseminations.* For most strains an average semen yield per male would fall between 0.7 and 1.0 ml. per collection. A typical range is 0.2 to 2.0 ml. over a period of several months. With three collections per week, one group of single comb White Leghorn cockerels averaged 0.8 ml. of semen per collection or 2.4 ml. per male per week over a ten-week period.

The standard recommended dose of whole semen per hen is 0.1 ml. However, experienced operators obtain good results with half this dose, or 0.05 ml. A dose of less than 0.05 ml. results in lower fertility, and there is no advantage in inseminating with more than 0.1 ml. If semen is diluted prior to insemination, the 0.1 ml. level is recommended.

For maximum fertility the hen should be inseminated at seven-day intervals. The average duration of fertility following semen introduc-

tion in the chicken is, for most strains, between 12 and 14 days. Weekly insemination is needed to take care of hens with a less than average duration of fertility. If only one insemination is to be made, fertility of about 85 per cent may be expected for a period of ten days. Unpublished data of the authors indicate that duration of fertility declines as the laying year progresses, therefore, late in the year it may be desirable to inseminate every four or five days.

#### THE TURKEY

All the basic methods for inseminating turkeys are based on the original descriptions of Burrows and Quinn. It is not necessary to describe the reproductive system of the turkey because of its close similarity to that of the chicken. In general, it is more difficult to obtain semen from a turkey tom than from a chicken cockerel, more practice and skill are required on the part of the operator. However, it is still possible to do the job with the simple equipment listed for chickens. Because of the great economic importance of artificial breeding of the turkey, it is natural that much thought and effort have been placed on the development of labor saving methods and equipment. To illustrate the importance of this technique in the turkey industry, it might be pointed out that one West Coast breeder planned to inseminate 150,000 hens during the 1959 season.

*Management of the Tom.* Management of the tom is basically like that of the cockerel. First of all, toms should be segregated from the hens. If it is necessary to use toms for both artificial and natural matings they should be kept away from hens for 24 to 48 hours before handling. Because of their size as well as the fact that large numbers are needed in most commercial operations, toms are rarely kept in individual cages. In case toms are kept in cages, solid floors are better than wire floors because of the danger of foot and leg trouble developing as a result of the wire or slats. Darkened quarters are recommended for the handling operation in order to facilitate catching and to prevent undue excitement. Overexcited toms tend to struggle, are difficult to stimulate, and frequently contaminate the semen with watery fecal material. Fecal contamination may be minimized by removing both feed and water about four to six hours prior to handling. In hot weather, however, it may be dangerous to deprive the tom of water.

It has been observed by the authors that semen collections may

be made as frequently as five times per week for a two-week period early in the season with no adverse effect on fertility, and only a slight decrease in volume of semen collected. However, the work of Lorenz *et al.* (1955) suggests that this practice may be harmful if followed for very long. Their results suggest that regular intervals of two, three, or seven days between collections are all satisfactory in respect to semen yield. In fact, yields were observed to decrease when the interval between collections exceeded one week (Lorenz *et al.*, 1956).

The turkey is still a seasonal breeder and much influenced by light. It is a common practice to use artificial lights to produce late winter and early spring poults. Once the toms have been stimulated by extra light early in the season, care must be taken that they remain on this same light ration until the natural daylight hours are equal to the artificial ones. If lights are cut out too soon or by accident, semen production not only ceases but will not return for an extended period.

**Collecting Semen from the Tom.** Basically the massage is the same as was described for the cock, but some slight modifications are helpful when dealing with difficult toms. Starting on the left side of the tom, place the left hand on the back of the bird about six inches in front of the tail, with all fingers on the right side and the thumb on the left side. Slide the hand firmly toward the tail, and as the tail is reached, slide the thumb around to the fleshy back portion. Next lift the tail up and back toward the front end of the bird. All of this is but part of one continuous motion, and a dozen or so may be necessary to bring about a response. A response is defined as partial protrusion of the papillae; trained toms usually respond after a single massage. Meanwhile, with the right hand, the operator should be massaging the abdomen along the underside of the pubic bones. After a partial response, he moves his left hand so that the palm is against the underside of the fleshy tail region, with the index finger and thumb positioned on each side of the vent. He should exert constant pressure with the thumb and finger toward the front end of the bird to aid in bringing about protrusion of the papillae. Milking should be started as soon as the papillae are fully protruded. As with the chicken, the right hand is used in collecting the semen.

The collector should have a soft, clean cloth available at all times so that he can clean away fecal material in the area of the papillae. The cloth may be held in the right hand during the massaging and

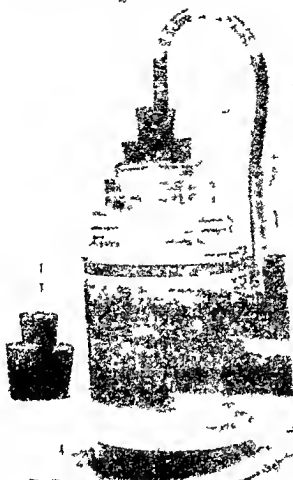


Figure 47 An aspirator type of semen collector which sucks the semen into a tube immersed in a vacuum bottle. The water in the bottle should be kept at a temperature of 75° to 85° F. (Courtesy Turkey World)

used quickly when needed. This practice is valuable in securing semen samples free of fecal material.

The one man technique of Gabriel (1957) may also be used with turkeys—using either a metal killing funnel or a wooden holding stand with straps or special leg holding devices. The authors believe that the first practical one man turkey inseminator was developed by Edward McGarry, a Massachusetts turkey breeder. For many years he has worked alone in collecting semen by means of tipped killing cones and then inseminating the hens with the same equipment.

Since a time interval of as much as one hour may exist between the initial semen collection, when the semen from many males is mixed, and the insemination of the last hen, temperature control of the holding-bottle is frequently of value. This is particularly true when the air temperature is low—which is the case at insemination time in many parts of the country. Most of these devices are simply vacuum bottles with a fitted rubber stopper through which the holding-tube extends. An example of this is shown in Figures 47 and 48. The water in the vacuum bottle should be between 75° and 85° F. for the average operation in which the total collection and insemination interval is less than one hour.

An aspirator type of collector that replaces the collection funnel and the need of shifting semen to the holding tube has been developed and is used widely on the West Coast (see Figure 48). One

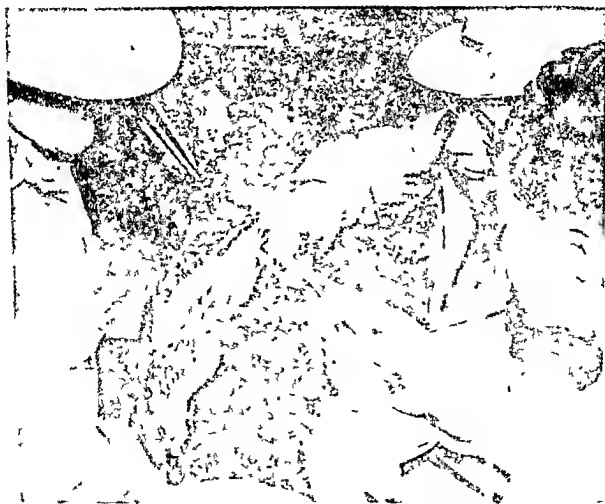


Figure 48 Collecting semen by the aspirator method eliminates the need for a collection funnel and mechanical moving of semen to the holding tube following each collection. (Courtesy Turkey World)

operator stimulates semen flow while the second runs the aspirator which sucks up the semen and draws it into the holding tube. The holding tube is suspended in a vacuum bottle (see Figure 47). This piece of equipment can be assembled from some glass and rubber tubing, a couple of rubber stoppers, and a vacuum bottle. It saves time, but there is the danger of contamination with urates and feces.

*Inseminating the Turkey Hen* The basic problem is to apply sufficient pressure on the soft abdominal area so as to cause eversion of the oviduct. In the original method a helper holds the hen in his lap and applies pressure on the abdomen while the inseminator holds her legs. Following eversion of the oviduct the syringe should be inserted to a depth of approximately one inch. A faster and easier way than this is one in which the hen is placed head first through the helper's legs and held in position by the thighs. The holder can apply considerable pressure with his legs, while he is also applying abdominal pressure with his hands (Figure 49). The inseminator or someone else should hold the hen's legs during the operation to discourage her from struggling and possibly injuring herself.

A number of holding devices, including a variation of the funnel method (as shown in Figure 50), have been developed in an attempt to save both time and labor. Another type is a series of killing funnels built into a wooden frame. The authors have found that the devices which hold the hen nearly parallel to the ground are superior to those which hold the bird in a perpendicular plane. In this latter position there is a tendency for watery droppings to run into the oviduct along with the semen. Figure 51 illustrates a desirable holding position.

Insemination should be done late in the afternoon to minimize the chance of finding hard shelled eggs in the uterus. Some of the large turkey breeders do not bother to palpate for presence or absence of hard shelled eggs in the uterus; they feel that a saving in time is more valuable than the slight decrease in fertility that results when some of the hens carrying hard shelled eggs are inseminated.

Many turkey hens are inseminated shortly before laying their first egg and in such cases a membraneous tissue, the hymen, is observed across the opening of the oviduct. This is normally broken either by the treading of the tom during the natural mating act or during the passage of the first egg. If the oviduct is easily everted, this mem-



Figure 49. Inseminating the turkey hen. The assistant holds the turkey firmly between his thighs with its head foremost and its wings free to envelop his thighs. His hands are thus free to apply abdominal pressure and to evert the oviduct. The inseminator is then ready to insert the syringe of semen.

brane may be broken and the hen inseminated with good results. Most of these membranes can be broken easily with the end of the syringe, but there is individual variation between hens.

Several types of automatic syringes which eliminate the necessity of measuring doses have been devised. Some are modified dental syringes, like the one described by Thumin (1951). This one has an extension on the outer piston rod fitted with a returner spring and a



Figure 50. Use of funnel-shaped holders for female during the insemination process. This particular model has a heavy base for stability but can be moved from one location to another without difficulty (Courtesy Turkey World.)

dose unit on the piston inner rod within the barrel. A more elaborate type has been designed by Precision Devices, Guilford, Connecticut (see Figure 52). It will deliver one dose of semen with each pull of the trigger. There is also a warming device which fits on the gun and keeps the semen at the right temperature during the operation.





Figure 51 A turkey inseminating team in action. Note angle of female on the holding table and points of pressure being applied by white-gloved helper (Courtesy Turkey World)

**Semen Yields, Dosages, and Intervals Between Inseminations**  
Semen yield per tom per collection varies with the strain, but an average figure is between 0.15 and 0.30 ml. Note that the yield from a tom is considerably less than that obtained from the chicken male. Sperm cell concentration is much higher in turkey semen than in

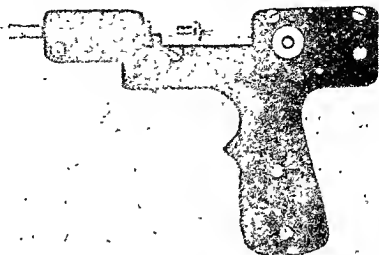


Figure 52. An automatic inseminator which delivers a measured amount of semen with each pull of the trigger. (Precision Devices, Guilford, Connecticut.)

that from chickens and in addition is more viscous and creamier in color. Semen yield is greatest in the middle of the breeding season and poorest at the end of the season. The authors have found that up to 7 or 8 per cent of all toms do not yield any semen even though they respond readily to repeated collection attempts.

A dosage of 0.025 to 0.035 ml. of semen per hen will give satisfactory results if the technique is good. Some breeders increase the dose to 0.05 ml. late in the breeding season, but there is no experimental evidence to substantiate this practice. Early in the season the average duration of fertility is between 40 and 50 days, but there is a gradual decline as the season progresses. A flock fertility of 85 per cent over a 30-day period has been observed following insemination early in the season. At first it is well to inseminate the hens every three or four weeks. By the third month the interval should be shortened to two or three weeks, and even less than this if the season extends to five months.

#### DUCKS AND GESE

As early as 1934, Serebrovsky and Sokolovskaya secured semen from a gander by electrical stimulation. Watanabe (1957) has also

been successful with this technique. In addition, a manual massage method has been described by Johnson (1954) and Watanabe and Sugimori (1957).

**The Reproductive Organs.** The reproductive organs of ducks and geese are very similar, but both differ in several respects from those of chickens and turkeys. A major difference is the presence of a well-defined penis in the drake and gander. The penis becomes quite enlarged during the breeding season and supplies definite evidence for sex determination in many varieties of geese. Just prior to natural mating, the waterfowl penis is extruded. Since the oviduct of ducks and geese cannot be artificially everted, a different technique for depositing the semen into the vagina is required.

**Collecting Semen from the Drake or Gander.** The manual massage method has been described by Johnson (1954) and is essentially similar to that used for chickens and turkeys. It would be repetitious to repeat this description, and the reader is referred to the two-man technique used for turkeys. Figure 53 shows a semen collection from



Figure 53. Collecting semen from the gander. Note the protruded penis and the collection tube

a protruded penis of a gander Semen is released at the base of the penis and normally runs in a canal the length of the organ Semen may be collected anywhere along the canal or from the end of the organ What has been written here for the gander applies equally well to the drake Handling must be gentle in order to avoid bleeding, and fecal contamination can be minimized by removing feed at least six hours prior to handling

An electroejaculation technique for drakes has been described by Watanabe (1957) giving better results than the manual massage method One electrode is placed on the skin in the sacral region and the other one inserted in the vent An alternating current of 30 volts and 0.06 to 0.08 amperes is applied for three seconds at five-second intervals—repeating three to five times

*Inseminating the Duck or Goose.* Since the oviduct cannot be everted, it is necessary to inject the semen directly into the vagina Johnson (1954) perfected a method in which the first step is to locate the opening of the oviduct by palpating in a direction slightly downward and to the left in relation to the vent opening After the oviduct is located, a 2.5-inch glass tube, attached by a short piece of rubber tubing to a standard tuberculin syringe, is guided into it Watanabe (1957) made use of a modification in which a metal speculum is inserted after the oviduct has been located Then a glass pipette containing the semen is inserted into the vagina by passing it through the speculum

*Semen Yields, Dosages, and Intervals Between Inseminations.* Semen produced by drakes and ganders is more watery and has less color than that produced by chickens and turkeys Concentration of sperm cells per milliliter appears to be extremely variable on the basis of the few recorded data available Johnson studied a total of 18 ganders and secured semen from only 12 of them Six of these were used extensively, and their average yield ranged from 0.05 to 0.60 ml per collection As might be expected, the best responses and yields occurred during the height of the breeding season Average yields of semen from common drakes have been reported as 0.23 ml (Watanabe and Sugimori, 1957) and 0.32 ml (Onishi, Kato, and Futamura, 1955) by the manual massage method, and as 0.33 ml by electroejaculation (Watanabe, 1957) Onishi and Kato (1955) report a larger yield of 0.82 ml from Muscovy drakes

There is not much information available on the optimum amount

of semen to use for insemination. Johnson used 0.05 ml. of whole semen with geese, and Watanabe used 0.30 ml. of whole semen diluted 1:10 with saline, or 0.03 ml. of whole semen, per insemination.

Johnson (1954) reported 9.7 days as the average duration of fertility in geese when figured from the day of insemination; however, the average was only 6.1 days following the laying of the first fertile egg. It should be noted that chickens, turkeys, and possibly ducks are extremely fertile on the second day following insemination, whereas the nonfertile period following insemination in the goose seems to be much longer—3.6 days in Johnson's study. It would appear that geese should be inseminated once a week. Watanabe and Sugimori (1957) reported common ducks as having an average duration of fertility of eight days. On the basis of the information available, ducks should be inseminated every five or six days.

#### DILUTION OF AVIAN SEMEN

It is not recommended that diluents be used in commercial programs of artificial insemination of poultry. Avian semen seems to be more susceptible to damage by artificial diluents than is the semen of many domestic animals. Bull semen diluents, such as egg yolk-phosphate and citrate buffers, are not satisfactory diluents for chicken semen. Sperm life is sustained for extended periods in the upper region of the avian oviduct where albumen is secreted, and yet egg albumen has been found to be a poor diluent. Certain albumen proteins, however, have shown some promise when added to diluents, according to Lorenz and Tyler (1951). It is frustrating to review the literature concerned with studies of avian diluents and extending fluids. Some investigators have failed to consider the time factor between dilution and insemination. If a diluent has a deleterious effect on the sperm cells, then the length of time that the sperm are in contact with the diluent is very important. Once inseminated, the diluting medium might be expected to have but little effect on the sperm. For example, in an experiment conducted by one of the authors, whole chicken semen and chicken semen serum were found to be excellent turkey semen diluents when used within a few minutes following mixing. However, when the diluted semen was held for half an hour before inseminating, it proved to be significantly inferior to undiluted semen.

**Chicken Semen** To date the best diluent for chicken semen has been found to be chicken semen serum (Munro, 1938, Weakley and Shaffner, 1952) In fact, Weakley and Shaffner used a 1:10 dilution rate without adverse effects, although higher dilutions were deleterious In cases of reduced fertility following the use of diluents, the diluents themselves must be harming the sperm cells If the diluted semen is not held too long before insemination, there are a number of solutions that may be used Some that do not reduce fertility more than 10 to 20 per cent are Ringer's, Locke's, and Tyrode's (see Table XIII) It is advisable to use chemically pure salts and to ad-

TABLE XIII SOME SOLUTIONS THAT CAN BE USED  
AS AVIAN SPERM DILUENTS

Ingredients	Grams per 1 000 ml of distilled water		
	Ringer's	Locke's	Tyrode's
NaCl	0 00	9 00	9 00
KCl	0 30	0 24	0 20
CaCl <sub>2</sub>	0 25	0 42	0 20
MgCl <sub>2</sub>	—	—	0 10
NaHCO <sub>3</sub>	0 20	0 20	1 00
Dextrose	—	1 00	1 00

just the pH to approximately 6.6 to 6.8, according to data published by Bogdonoff and Shaffner (1954)

**Turkey Semen.** The best turkey semen diluent used at the Massachusetts Station has been Earle's solution, but it resulted in approximately 15 per cent poorer fertility than did undiluted semen This was also definitely inferior to the results of van Tienhoven and Steel (1957), who used Tyrode's solution (pH 6.7) and homogenized, pasteurized whole milk (heated at 85° to 95° C for ten minutes) When used at dilutions of 1:1 and 1:3, the resulting three-week fertility was approximately equal to that for the undiluted semen Variations in time between dilution and insemination of 13 to 106 minutes did not affect fertility

**Duck Semen.** Watanabe (1957) diluted drake semen at a rate of 1:10 with saline solution and got satisfactory results More data are needed before it is safe to conclude that drake sperm are more resistant to salt solutions than are chicken sperm

## STORAGE OF AVIAN SEMEN

No completely satisfactory method for preserving avian semen for any length of time has yet been devised. Recent findings, however, are encouraging.

**Chicken Semen.** Many reports show a decrease in fertility following the use of semen stored for as long as two hours at various temperatures. Perhaps the best recent results with whole semen are those of Garren and Shaffner (1952), who were able to maintain fertility at 62 per cent—22 per cent below that of unstored semen—after a six-hour holding period at 48° F. In addition, they found that storage reduced the duration of fertility in all stored groups. Fertility was improved when the dose was increased from 0.10 to 0.20 ml. and the interval between inseminations was increased. If whole semen is to be stored for more than one hour, the temperature should be reduced gradually to 48° to 57° F.

Recently F. H. Wilcox of the University of Maryland has devised an interesting new method of holding semen. By use of his method he has been able to send samples of chicken semen by air to Scotland and to Israel, with the resulting average fertility of 37 per cent for the first week following insemination. These results are very interesting in view of the fact that the time lapse between collection and insemination was about 37 to 38 hours, plus exposure to the rigors of air shipment. Wilcox's method involves the use of two different diluents—one for diluting and storing the semen and the other for reconstruction before insemination. Immediately following collection, diluent A (16.34 gm. of  $\text{Na}_2\text{HPO}_4$ ; 5.16 gm. of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ; 100 mg. of terramycin; and 1,000 mg. of dihydrostreptomycin, all added to 1 liter of distilled water) is added at a 1:10 dilution rate; diluted semen is stored at 50° F. Following storage, the sample is centrifuged at approximately 1,200 R.C.F. for ten minutes and the clear fluid is poured off, leaving only sperm. Diluent B (made by adding 1 ml. of a solution containing 60 mg. of fructose per ml. to 14 ml. of a buffer solution containing 5.16 gm. of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  and 16.34 gm. of  $\text{Na}_2\text{HPO}_4$  per liter) is added to the sperm in an amount which will reconstitute to the original volume of the semen sample before the first dilution. The mixture should be stirred with a small glass rod until the sperm are well mixed with the diluent, and then inseminated at once.

**Frozen Chicken Semen** There is now considerable interest in the possibility of successfully storing chicken semen in the frozen state. Shaffner (1942) was able to show some fertility, but no hatchability, after quick-freezing semen at  $-76^{\circ}\text{C}$  and storing it for as long as 14 months. Later, Smith and Polge (1950) found that by adding 15 per cent glycerol before freezing at  $-79^{\circ}\text{C}$ , the revived sperm showed a high degree of motility but gave no fertility. Polge (1951), following this same technique, removed the glycerol by dialysis with Ringer's solution and obtained 54 per cent fertility during the week following insemination. Since Allen and Bobr (1955) have shown that fertility from semen glycerol mixtures can be obtained by inseminating directly into the uterus, some combination which includes freezing at  $-79^{\circ}\text{C}$  with 15 per cent glycerol, followed by dialysis and intrauterine inseminations, should result in the satisfactory use of frozen semen.

**Turkey Semen** What has been described for the chicken is equally true for the turkey, although there are some indications that whole turkey semen is more resistant to storage than is chicken semen. Carter *et al* (1957) stored whole turkey semen at  $57^{\circ}\text{F}$  for zero, two, four, and six hours with resulting fertilities of 93, 80, 72, and 55 per cent, respectively. There were no significant adverse effects of holding time on hatchability. Harper (1955) found that undiluted turkey semen could be held, at the beginning of the breeding season, for as long as four hours at  $55^{\circ}$  to  $60^{\circ}\text{F}$  without affecting fertility. A holding time of only one hour, however, resulted in a decrease in fertility as the breeding season progressed. At the present time it would not appear desirable to hold turkey semen for more than one hour prior to insemination. The correct holding temperature probably is between  $75^{\circ}$  and  $85^{\circ}\text{F}$ .

#### LABORATORY EVALUATION AND FERTILIZING ABILITY

No single laboratory measurement of semen quality has been found that will accurately predict fertilizing ability of a semen sample. More research work is needed in this area. From the data of Parker *et al* (1942) and Shaffner and Andrews (1948) it seems apparent that neither volume nor sperm concentration is related to fertility, except when total sperm number introduced falls below 100 million (Munro 1938). There is some positive relationship be-



tween high initial motility and good fertility. Initial motility must be scored arbitrarily, with rapidly swirling samples scored highest and samples showing nothing but individual cell motility scored lower. Parker found that fertility decreased with increasing numbers of abnormal sperm types, as observed in semen smears on glass slides. Shaffner and Andrews (1948) found that the persistence of sperm motility in semen stored at 4° C. was positively correlated with fertility. Bogdonoff and Shaffner (1954) have described the methylene blue reduction test and reported that the shorter the time required to reduce methylene blue to yellow, the better the fertility obtained.

By way of summary, it seems that initial motility score is the easiest measure to obtain since the only equipment needed is a microscope. The methylene blue reduction test requires special equipment and cannot be read for at least half an hour. Determinations of the degree of motility following storage and the percentage of abnormal sperm are valuable in research studies but are too time consuming to be of much practical value.

#### PRACTICAL APPLICATIONS

During recent years artificial insemination has become an important commercial practice in the turkey industry. It was mentioned earlier, and bears repeating, that one West Coast breeder planned to *inseminate 150,000 turkey hens artificially during the 1959 season*. McCartney (1951) reported that by instituting artificial insemination he increased the number of poults per breeder from 42 to 55 in a medium-sized White strain. The authors have obtained an average of 90 per cent fertility for an entire breeding season by artificial insemination alone. Many turkey breeders use artificial breeding only to insure high fertility at both the beginning and the end of the breeding season. It is fair to state that fertility in heavy strains may be usually stepped up by 15 to 20 per cent if artificial insemination is used to supplement natural matings.

Because of the efficiency of natural matings in the domestic fowl, there is a decided limit to the use of artificial breeding in this species. There are, however, occasions when it can be employed to good advantage. For example, if birds are kept in laying cages, it is a very efficient way of securing fertile eggs. By using 30 cockerels, handling them once daily for six days each week, and pooling the

semen, two men working two hours per day can inseminate 250 hens or take care of a flock of 1,500 hens. On this basis it is possible to produce approximately 5,000 chicks per week from this flock with an output of 24 man hours of labor.

Artificial insemination could be of considerable value to the pedigree breeder who keeps the breeding hens in individual cages. Some years ago a commercial breeder in New Jersey produced over 6,000 pedigreed chicks from 144 hens and 26 cockerels. His labor output for the ten week period was 40 man hours of skilled labor, that is, labor required to artificially breed these birds.

Pedigree breeders who wish to change breeding males in the middle of the breeding season find that artificial insemination is worth the little extra work required. The breeder can save an entire week in his operations at a time when a week is of considerable value. The procedure is to remove the old male from the breeding pen seven days before it is planned to stop saving hatching eggs. During these last seven days the hatching eggs will be highly fertile as a carry-over from the old male. Then on the morning of the seventh day following removal of the old male, all hens are inseminated with semen from the new male. It is safe to begin to save hatching eggs which can be attributed to the new male seven days following the artificial insemination. Actually the error would be slight if one began to save hatching eggs only three days after insemination, because fresh sperm almost always supersede the older sperm. As soon as the artificial insemination is completed, the new male should be given his liberty in the breeding pen so that he may begin to mate naturally. Credit for developing this time saving technique goes to D. C. Warren, formerly of the Kansas State College. This procedure is not so practical with turkeys, since, as Kosin and Wakeley (1950) found, older sperm are not completely replaced by new sperm for some time.

It has been claimed that artificial insemination can be used successfully in prolonging the effective reproductive life of aged sires which have already proved their breeding worth. To date, however, no data are available to verify this claim.

With artificial insemination it has been possible to make crosses that do not occur naturally. For example, it is known that a pheasant cock sometimes will mate naturally with a hen of the domestic fowl, but the reciprocal cross does not occur in nature. Artificially, it has

been possible to cross successfully the male of the domestic fowl with a ring-necked pheasant hen. Similarly, a successful cross between the domestic fowl and guinea fowl has been reported. One writer has claimed that semen has been artificially collected from males of the following: peacock, finch, and canary. In studies of the inheritance of body size in the domestic fowl, it has been possible to cross bantams with normal-sized fowl by using artificial insemination, whereas with natural matings such a cross is unpredictable because of extreme differences in body size. Attempts have been made to cross the turkey and the domestic fowl—using artificial insemination—but no viable offspring have been reported. It is a simple matter to make this cross reciprocally.

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# Swine

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JOHN AAMDAL

The artificial insemination of swine is still in an experimental stage, however, it is already of practical commercial importance in some countries

## THE NORWEGIAN ARTIFICIAL VAGINA

The semen of the boar is readily collected with an artificial vagina, and different types of artificial vaginas have been devised (Aamdal, Hogset, Sveberg and Koppang 1958, Ito *et al*, 1951a, McKenzie, 1931, Polge, 1956)

The Norwegian type of artificial vagina, shown in Figures 54 and 55, has been used successfully in artificial insemination over a long period. The length of the vagina is about 18 cm, and it has the same diameter as the model used for the bull. It contains two inner tubes, one for air and one for water, since the valve of the air pump becomes clogged if water and air are in the same compartment. Experience has shown that the boar prefers smooth inner tubes.

An inner tube, with the rough surface turned in, is used as a funnel. The semen is collected in a 500-ml plastic bottle, which has a double lid perforated with 2-mm holes. The gelatinous part of the semen is retained as the rest passes through the lids, and no further filtration of the semen is necessary. The vagina is warmed with 300 ml of water at 50° C (122° F), then lubricated with

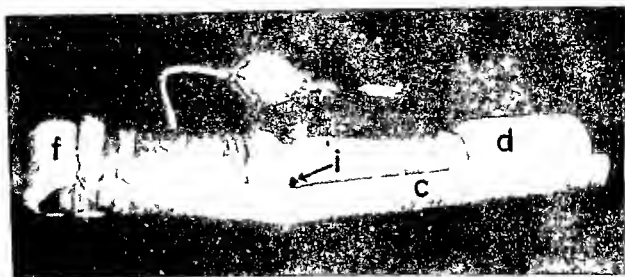


Figure 54 The Norwegian type of artificial vagina for the boar. Notice the foam rubber piece, *f*, covering the entrance the hole *i* in the funnel near its fastening to the vagina and the supporting splint *c* holding the collection bottle *d*.

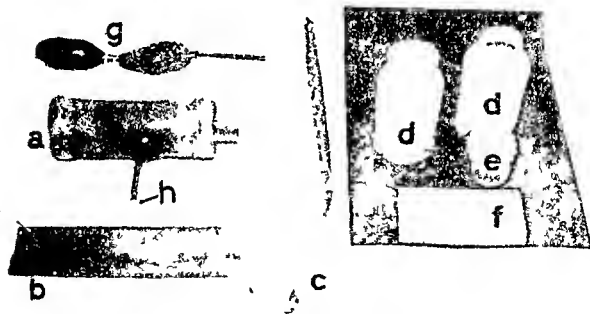


Figure 55 The Norwegian artificial vagina for the boar disassembled. The vagina *a* is 18 cm long with the same diameter as the model used for the bull. The inner tubes 28 cm long are used as a funnel *b*. There is a metal supporting splint *c*, 46 cm long and 5 cm wide. The 500 ml plastic bottles *d*, have double plastic lids *e*, perforated with 2 mm holes. The foam rubber piece *f* is 2 cm thick, 7 cm wide, and 14 cm long and has a Y-shaped slit (0.8 cm in each *d* direction) for the penis. The air pump *g* is attached to the tube *h* on the vagina.

sterile vaseline and air pumped in. The pump has a valve to permit release of air in case the pressure is too high.

The foam rubber piece, with its Y shaped slit, is fastened by means of a rubber band over the entrance of the vagina, and the vagina is attached to a metal splint in the same way. The plastic bottle is supported by means of a rubber band at the other end of the splint. A hole in the funnel, near the vagina, permits escape of preputial secretion during semen collection, and thus prevents it from mixing with the semen. Also, the splint is bent up to prevent flow from the vagina into the bottle.

The foam rubber piece is designed to give the penis a mechanical cleansing as it passes through it and also—like the hole in the funnel—to prevent in some measure, the flow of preputial secretion into the vagina and the semen bottle. Except for the foam rubber piece, which is used only once, the entire apparatus ought to be boiled after each collection.

#### THE DUMMY SOW

It is possible to collect semen from a boar when he is mounted on a sow in heat. It is more convenient, however, to make collections when the boar is mounting a dummy sow.

Different types of dummy sow with more or less complicated construction have been devised (Gotze 1949, Ito *et al*, 1951a). In Figure 56 is shown a dummy sow which has proved satisfactory both from the operator's and boar's point of view and it is also easy to keep clean. Its frame is made of  $\frac{3}{4}$  inch galvanized iron tubing stuffed to a proper shape and covered with a plastic cloth. During the collection of semen the dummy may be soiled with secretion from the prepuce, and bacteria can be transmitted from one boar to another. Therefore washable covers are used and changed after each collection.

The dummy is fixed to a light wooden platform so that the boar keeps it in place by means of his own weight. The hindmost part of the platform is covered by a rubber mat so that the boar will stand steady, and a block on the front part of the dummy prevents the boar from getting too far forward. The hind legs of the dummy sow may be lowered or raised according to the height of the boar being used. To ensure a convenient working position for the operator,



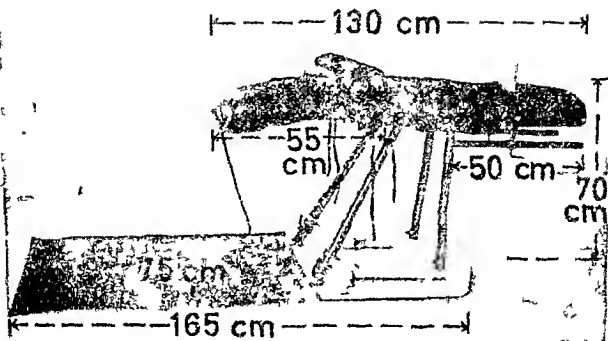


Figure 56 The dummy sow, made of galvanized iron and stuffed, has a changeable cover. The adjustable wooden block top right prevents the boar from getting too far forward during collection of semen.

it is important that the hind legs of the dummy be placed well forward

#### SEMEN COLLECTION PROCEDURES

When the boar mounts the dummy, the operator guides the penis into the artificial vagina. If the artificial vagina is the Norwegian type, he threads the penis through the Y shaped hole in the foam rubber piece. The operator then sits down on a stool on the right side of the dummy, with the vagina in his left hand and his right hand grasping the funnel (Figure 57). The operator then tries to catch and hold the penis, through the vagina, with his right hand. As a rule, the boar will move the penis forward and backward several times while the operator is trying to get a firm hold on it.

As soon as he has the penis fixed in his hand, the boar will begin to ejaculate (Figure 58), and an assistant should detach the plastic bottle from the supporting splint so that the semen can flow directly into it.

During the attempts to catch the penis the vagina must always be held in such a way that preputial secretion will not flow down into

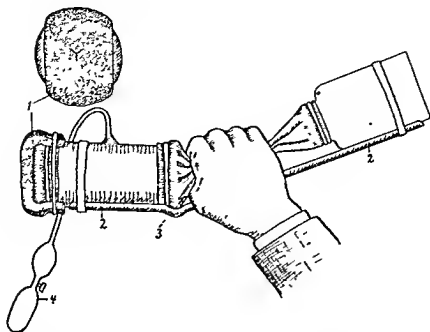


Figure 57. Method of grasping the penis prior to ejaculation. 1. Slit in foam rubber piece. 2. Bent splint. 3. Hole in funnel. 4. Air pump.

the bottle. Usually, some of the secretion that has passed the foam rubber piece will flow out through the hole in the funnel.

### SEMEN CHARACTERISTICS

Boar semen consists of three fractions: a prespermial fraction, mainly gelatinous material from Cowper's glands, the sperm-rich fraction, and the sperm-poor fraction, mainly gelatinous material and fluid from the accessory glands. It is presumed that the main function of the gelatinous material is to prevent the backflow of semen during and after coitus.

Table XIV shows the volume and density of semen from three different boars used in artificial insemination.

TABLE XIV VOLUME AND DENSITY OF BOAR SEMEN

Boar	No of examined specimens	Total specimens (c.c.)	After gelatinous material removed (c.c.)	Density (1,000/ cc. H <sub>2</sub> O.)	Gelatinous material (c.c.)	Gelatinous material in whole specimen	Total no sperm in specimen (in billions)
A	14	293	225	253.4	68		64.89
B	28	271	205	229.6	66	22.2%	47.07
C	29	214	173	256.6	41	24.3%	44.39
Mean	91	261	202	261.5	59	22.6%	52.82

The density and total number of sperm per ejaculate are subject to great variation depending on frequency of collection and individual diversity.

The semen of the boar contains a high number of bacteria, on the average 180,000 per ml. if an artificial vagina of the ordinary type as designed for the bull is used (Aamdal, Högset, Sveberg, and Koppang, 1958; Tanaka *et al.*, 1951). Since the semen contains only a small number of bacteria as it leaves the tip of the penis, the major part of the bacteria comes from the boar's prepuce (Aamdal, Högset, Sveberg, and Koppang, 1958; Koppang and Filseth, 1958). If the Norwegian type of artificial vagina is used, so as to prevent the preputial secretion from flowing into the semen bottle, the number of bacteria in the semen is decreased to 10,000 per ml. on the average.

The microscopic determination of the quality of boar semen is best made with diluted semen and by means of a phase contrast microscope, 200 diameters magnification. The temperature on the object table should be 40° C. (104° F.). Furthermore, the sperm needs three to five minutes to reach maximum motility.

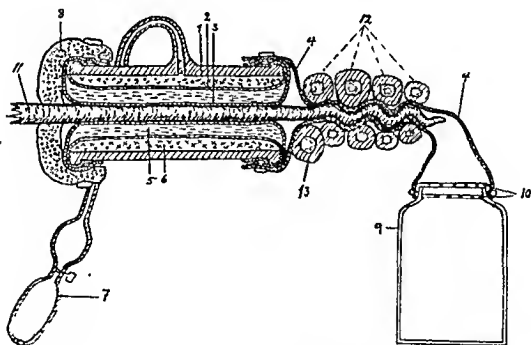


Figure 58. Cross section of the artificial vagina during ejaculation. 1. Outer wall of the vagina. 2. Inner air tube. 3. Inner water tube. 4. Funnel. 5. Water. 6. Air. 7. Air pump. 8. Foam rubber piece. 9. Plastic bottle. 10. Double perforated lid. 11. Penis of boar. 12. Cross section of fingers of right hand. 13. Cross section of thumb.

### SEMEN DILUTION

Through the years, various diluents have been tried for bull semen, and many of these have also been tried for boar semen. When diluted with 3 per cent glycine and 30 per cent egg yolk (Polge, 1956), or 9 per cent skimmed milk powder (Melrose), the boar semen may be successfully stored at 41° F. The 3 per cent sodium citrate—30 per cent egg yolk is a very good diluter for boar semen when stored at 15° C to 20° C (59° to 68° F.) As this temperature also is highly suitable for growth of bacteria, it is important that semen collections be as clean as possible and that antibiotics be added at the rate of 1,000,000 international units of penicillin and 1 gm of dihydrostreptomycin per 1,000 ml sodium citrate. Boar semen stored in sodium citrate diluter at 15° C to 20° C (59° to 68° F.) shows good survival, and the conception rate is the same up to 30 hours after collection.

The minimal volume needed per insemination is found to be 50 ml of diluted semen. In artificial insemination procedures wherein some waste of semen is likely to occur, a larger volume is required. A dose of 100 to 150 ml of diluted semen per insemination has given good results and seems to be warranted.

Since there is a great variation in density of boar semen, the dilution rate must depend upon the density of the sample. At present there is little knowledge about the effect of increased dilution of boar semen on the conception rate. Dilution up to 4 billion sperm per insemination dose, that is, 8 to 15 doses per ejaculate, has been used with good results.

### ESTRUS IN SOWS AND TIME OF INSEMINATION

The length of estrus in sows has been found to vary with the breed (Burger, 1952). The mean length of estrus in Large White sows is 48 hours and in Large Black, 60 hours. Ovulation occurs about the eighteenth to the thirty-sixth hour of estrus in Large White and from the forty second to the fifty fourth hour in Large Black sows.

Two to three days before estrus, sows and gilts show signs, such as reddening and swelling of the vulva.

Breeding experiments show that in natural mating the same percentage of pregnancies is obtained by mating at 0, 12, and 24 hours after onset of estrus in Large White, and at 0, 12, 24, and 36 hours in Large Black sows. At matings later in the estrus, the conception rate was much lower.

As to the optimal time for artificial insemination, no report is at present available. It is surmised that insemination during the first half of estrus gives the best conception rate, as in natural mating. Experience from artificial insemination on a big scale seems to confirm this supposition.

#### INSEMINATION PROCEDURES

The cervix of the sow is long (Figure 59), and the canal is curved in the upper part and so narrow that it is impossible to pass any kind of instrument through it without damage to the tissues.

In order to squeeze the semen through the narrow part of the cervix and into body of the uterus different types of apparatus have been devised, such as a rubber tube, an ebonite tube, and a glass tube. In Figure 60 a new type of equipment is shown. The equipment consists of a plastic tube about 50 cm. long with a diameter of 8 mm. About .2 cm. from the tip a plastic cuff (diameter,

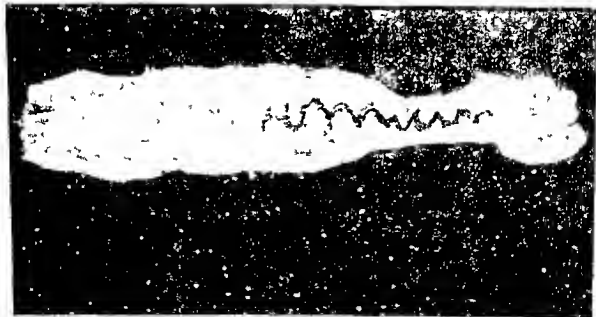


Figure 59. Longitudinal section through the vagina and uterine cervix of a sow. The curved course of the cervical canal is stained.

4 cm ) is fixed, and this may be inflated by means of a pump. When the cuff is inflated, it stoppers the cervix, so that the semen does not flow back into the vagina during insemination (Figure 61). The cuff has about the same functions as the gelatinous part of the semen is presumed to have in natural mating. The 150-c.c. plastic bottle, also used for transportation of semen, is coupled to the plastic tube by means of a rubber connecting piece with attached head.

When the operator is ready to make an insemination, both the plastic tube and cuff should be lubricated with liquid paraffin and the tube pushed as far into cervix as possible (Figure 61). The cuff is then fully inflated, the semen bottle is coupled to the plastic tube, and the semen slowly injected. In case the semen leaks out through the vagina the cuff must be deflated, the tube pushed further in the cervix, and the cuff once more inflated.

#### PRACTICAL ASPECTS

Since boar semen varies widely in quality, the semen of prospective sires should be examined before they are accepted in an artificial breeding program. The examination is best done at the breeding center. In natural mating a fairly high conception rate can result even if the boar's semen is of poor quality, but in artificial insemination the rate will be low. Therefore, a boar whose semen is not of good quality should be rejected for artificial breeding.

As a rule, there is no problem in collecting semen from a boar in a herd. If the hind part of the dummy is moistened with urine, the boar will mount.

In experimental work, boar semen has been collected at 48-hour intervals. However, practical experience in artificial insemination of swine seems to show that boar semen collection over a long period should not be made oftener than twice a week or three times a fortnight. Some workers presume that overfrequent collections will cause a decrease in semen quality and conception rate.

The big problem in practical use of artificial insemination at present is to detect the estrus in the sow and to get her inseminated at the optimal time that is during the first half of the period.

The signs of reddening and swelling of the vulva commence two to three days before estrus and there is an even transition from these symptoms to estrus. The typical proof that the sow is in estrus



Figure 60. Sow insemination equipment. The plastic semen bottle is attached to the insemination tube. The rubber pump is used to inflate the tip of the tube. Note the ready-to-use insemination tube, sterilized and inserted in a plastic stocking.

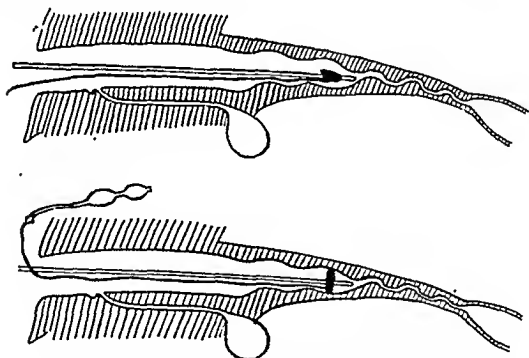


Figure 61. Longitudinal section through the genital organs of a sow, with the insemination tube pushed as far as possible into the cervical canal. In the second drawing, the plastic cuff at the tip of the tube has been inflated.

is that she "stands" for the boar. When no boar is available for testing the sow, the farmer himself has to find out if the sow is standing by trying to sit on her back.

To gain experience, the owner should follow the sow closely during one heat, noting the changes in symptoms; and he may inseminate in the next heat.

#### RESULTS WITH ARTIFICIAL INSEMINATION IN SWINE

Although good conception rates have been obtained in experimental work on the artificial insemination of swine, the rates vary greatly in general farm practice. In Norway, a total of about 7,000 inseminations are performed each year, representing about 12 per cent of all mating in swine. In 1,700 first inseminations over a six-month period, the conception rate, based on the number of farrowing sows, was 56 per cent. Semen was diluted at the rate of 30 million sperm per ml., and the diluter used was 3 per cent sodium citrate—30 per cent egg yolk, with added antibiotics. The diluted semen was stored at 15° to 20° C. (59° to 68° F.), and inseminations were performed by different veterinarians between 4 and 30 hours after collection. The insemination dose was 120 to 140 ml., that is, 8 to 15 doses per collection. Semen was shipped as much as 300 miles.

There was no significant difference in the size of the litter when litters resulting from natural mating and from artificial insemination were compared. In 3,000 litters resulting from artificial insemination in Norway, the average was 9.65 piglets per litter.

In France in 1961 over 11,000 sows were artificially serviced, with a conception rate of 70 per cent on first insemination. Semen was collected three times a week. In Holland, about 60,000 sows were inseminated artificially in 1962. (Dziuk, 1962.)

Semen can be shipped long distances with satisfactory results. In 1956, boar semen was sent by air from Norway to Beltsville, Maryland, and 24 sows were inseminated 30 to 40 hours after the semen was collected. Eleven of the sows (46 per cent) farrowed.

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## Dogs

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ELLIS P. LEONARD.

The first recorded instance of artificial insemination in the bitch was in 1780. Abbé Spallanzani is reported to have artificially impregnated a bitch at that time, and after 62 days she whelped three puppies, two male and one female. All three pups were said to resemble the male parent. From time to time other sporadic and successful attempts were made to inseminate bitches artificially. Most of the early work was of an investigational nature, and only in recent years has artificial insemination been practiced in order to effect certain matings that otherwise would be impossible. Even today the use of this procedure is not nearly so prevalent in the breeding of dogs as it is in the breeding of cattle and some of the other animal species.

Up to the present time the American Kennel Club has not encouraged the practice of artificial mating, although it will cooperate with the breeder if he wishes to employ the method or if he finds it necessary to do so. When a natural mating cannot be consummated, application for registration of a litter born as the result of artificial insemination will be considered by the American Kennel Club provided there is compliance with certain stipulations.

The American Kennel Club must be furnished with a properly completed official litter application form, an affidavit by the owner of the sire certifying that he witnessed the extraction of the semen and its insemination by a licensed veterinarian, identified by him in his affidavit, and an affidavit from the veterinarian that he made

the extraction and the insemination and that this was done in the presence of the sire's owner Both statements must identify the sire and dam by American Kennel Club name and number and must state the time and place of the mating

In the case of dog semen, no adequate controls have been set to govern its shipment Since it has been difficult to preserve the semen for any length of time, this natural control has somewhat limited the widespread distribution of semen from a given stud

#### ANATOMY OF THE DOG

Of all the domesticated animals, the dog probably has the simplest genital anatomy In the male, seminal vesicles and Cowper's gland are not present, and the only accessory sex organ is the prostate This is located at the neck of the bladder and completely surrounds the prostatic portion of the urethra, to which it is closely adhered The prostate empties into the urethra through many small openings in the urethral wall on either side of the urethral crest Hypertrophy of this gland is common in older dogs

The testes, epididymis, and ductus deferens are essentially the same as those of the bull The penis consists of a root attached to the ischium, a body, and a relatively large glans with a bulb at its caudal end The anterior portion of the body, and most of the glans, has a bone passing through it This os penis has a deep ventral groove through which the urethra passes

The mechanics of erection is not well understood in the dog The bulb of the glans becomes very large during erection and serves as the male part of the locking device which causes a tie during coitus

The reproductive organs of the bitch consist of vulva, vestibule, vagina, bicornate uterus, and ovaries The vulva, which lies some distance below the anus, is relatively small during anestrus During heat the vulva becomes engorged, enlarged, and as the proper time for mating approaches, changes in texture from a hard, rigid organ to one that is soft and spongy The vestibule is that part of the female genital canal which extends from the vulva to the urethral opening This part of the canal is nearly vertical in direction except during the copulatory act, when it may assume an almost horizontal position The vagina, uterus, and ovaries need no special description here

## ESTROUS CYCLE OF THE BITCH

The reproductive cycle in the bitch can be divided into four stages, that is, anestrus, proestrus, estrus, and metoestrus. This cycle is ordinarily completed twice each year, although there may be individual variations in the length of the cycle (172 days to 200 days).

Anestrus can be described as that period beginning at the end of regressive changes following one estrus and ending at the onset of proestrus. This period seldom exceeds two months. A vaginal smear made during anestrus is composed primarily of leucocytes and epithelial cells.

Proestrus is the period extending from the first appearance of a sanguineous discharge to the time of first acceptance of the male. This period may vary between seven and nine days but usually is about nine days. The vaginal smear during this period reveals many erythrocytes and cornified epithelial cells. Leucocytes are absent.

Estrus is the period from the first to the last acceptance of the male. The length of true estrus may vary between five and twelve days, with an average of about nine days. Ovulation usually occurs at about the second day of true estrus. The characteristic elements of a vaginal smear at this time are erythrocytes and disintegrating cornified epithelial cells. There are also large numbers of bacteria present.

Metoestrus is the regression period beginning at the end of estrus and extending to the resting or anestrus stage. This period is approximately three months in length. A vaginal smear during this period will show the return of leucocytes and the gradual change of cornified epithelial cells to those having the characteristics of anestrus.

## THE COPULATORY ACT

The dog is attracted to the bitch in estrus long before she is ready to accept him. In the normal bitch the acceptance period is only during true estrus. There may be considerable variation in the length of this period, in some cases nine or ten days and in others only a day or two. Some bitches will court or tease a stud a full day before they will accept him. The teasing period is likely to be prolonged in cases wherein the stud is not a particularly vigorous one.

A good, experienced stud may not allow any teasing but will mount and breed a bitch as soon as she is placed with him.

When the bitch is ready to accept service, she will turn her genitals toward the stud, draw the tail to one side, and extend the vulva in such a manner that the vestibule is in a somewhat horizontal position. She stands quietly for the male to mount and allows him ample time to make an entry. If the stud is exceedingly slow, the bitch will probably resort to teasing again in order to stimulate him.

As the stud mounts he protrudes the anterior portion of the glans and attempts to contact the vulva. When contact is made, the penis is thrust into the vestibule, the prepuce being pushed back of the bulb of the glans, and the bulb forced between the lips of the vulva. A series of short thrusting movements are then made, and it is during this part of the copulatory act that the true erection of the penis takes place. The entire glans, including the bulb, becomes greatly engorged and enlarged. The lips of the vulva grasp the penis just posterior to the bulb and thus effect a tie.

Ejaculation begins with the coital thrust, and the first phase is the emission of a clear, watery fluid which continues until erection is complete. At this point the thrusting movements of the stud cease, but ejaculation continues into the second phase. This ejaculate is a whitish, milky fluid which contains large numbers of spermatozoa. One can observe the ejaculatory action by noting the pulsations at the root of the penis. This phase may last from five to fifteen minutes. Frequently the stud dismounts during this phase and stands facing away from the bitch.

#### SEMINAL CHARACTERISTICS

Comparatively little work has been done with dog semen. Lambert and McKenzie (1940) report the volume of ejaculate to be 2 to 19 ml (most common volume 7 ml). Nooder (1950) places the volume at 1 to 20 ml and comments that volume may vary considerably in the same dog. The number of sperm per milliliter has been variously reported from 88 million to 588 million, and from 69 million to 1,726 million per ejaculation. Alifanov (1934) observed that sperm were produced continuously and that the normal amount was restored at intervals of 24 to 72 hours. Nooder states that in the dog, prolonged sexual rest has no effect upon the quality of the semen.

It was also found in studies by Boucher *et al.* (1958) and also in those made by Blackledge (1958) that there was no appreciable alteration in semen quality when collections were made as frequently as every other day for short intervals. Even when a stud dog is ejaculated daily for short periods, a 24-hour rest will usually provide sufficient rest for complete restoration of sperm reserves.

Color of the ejaculate may vary from light gray to white, and the consistency may range from watery to viscous. This variation in color may be due in part to the concentration of sperm in the semen sample.

Blackledge describes a method of calculating the number of sperm using a photometer. With this method, the semen is diluted 1 to 4 or 1 to 9 with a 2.9 per cent sodium citrate solution and the optical density determined with a Klett-Summerson colorimeter using a green filter. From this calculation, the total sperm per ejaculate and the total motile sperm per ejaculate can be determined.

Individual movements of the spermatozoa are intense in fresh samples, and mass movements also are sometimes seen. Nooder (1950) observed not more than 20 per cent morphological abnormalities in good sperm.

#### SEMEN STORAGE

Survival time for normal semen is about 21 hours. Bederke (1933) found that dilution with physiological saline reduced this time by 12 to 15 hours. He also found that dilution with dog serum reduced the survival time by half and that dextrose solution alone or with sodium phosphate buffer had an unfavorable influence. More recently it has been found that survival time can be extended to as much as 173 hours through the addition of such extenders as pasteurized milk or egg yolk-citrate. However, it has been found that dog semen is extremely sensitive to most extenders and actually survives best with one of the two just mentioned.

Harrop (1954a) uses the following method of preparing the milk extender: One-half pint of ordinary pasteurized milk is slowly heated to 92° C. and kept at that temperature for ten minutes; it is then allowed to cool to room temperature; 10 ml. are then carefully poured into a test tube, without disturbing the scum that has formed on the top of the heated milk. The second fraction of the

semen sample, after having been examined for motility, is slowly mixed with the heat treated milk at the rate of 1 part of semen to 7 parts of milk

Footc, using an extender consisting of 20 per cent egg yolk-citrate and 15 per cent glycine, was able to extend survival time to eight days. The criterion in this experiment was motility, and as yet the test for fertility of the sperm has not been carried out. In the case of the milk extender, Harrop and Leonard (Leonard, 1956) were able to impregnate a bitch with semen that had been preserved in milk for 140 hours and shipped by air from London to Ithaca, New York. She whelped five puppies.

#### INDICATIONS FOR INSEMINATION

Generally speaking, artificial insemination is resorted to in dogs when natural matings are impossible because of geographical distance between stud and bitch, or for physiological or psychological reasons.

In the dog, premature erection may prevent entry of the penis into the vagina. This may result if the stud is attempting to make entry on a small or immature bitch. An occasional stud may have premature erections habitually. Some studs, in which the semen may be normal, will show evidence of impotency through lack of sexual drive.

Artificial insemination becomes necessary if the bitch has developed a neurosis as a result of being trained as a watch dog, or if she has become thoroughly upset due to transportation. Bitches with vaginitis frequently will not conceive following a natural breeding but can be impregnated artificially.

#### SEMEN COLLECTION AND EXAMINATION

Equipment for collection of semen by one method consists of a sterile 20-ml glass syringe with a rubber adapter (Figure 62). Glass slides and a microscope should be available for examination of the semen. Whenever possible, collection and insemination should be done at one operation.

As the first step in artificial insemination, the bitch should be presented to the stud, and as soon as he shows interest, the collector



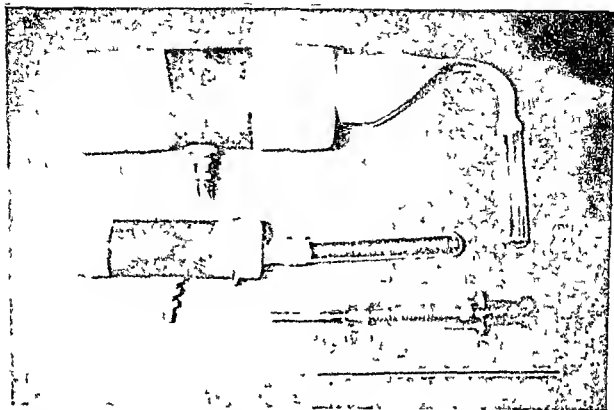


Figure 62 Two sizes of artificial vagina, a syringe with a rubber adapter used for the collection of dog semen, and an Inseminating tube

grasps the bulb of the glans in one hand with slight pressure behind the bulb. Having withdrawn the plunger from the syringe and doubled over the adapter to prevent leakage, he holds the barrel in his free hand and uses it for the collection of semen (Figure 63). He should take care that the penis does not enter the syringe, and in the case of shy breeding studs the syringe should not even be allowed to touch the penis. If the first part of the ejaculate is lost due to thrusting motions of the stud, this is of little consequence because the part containing most of the spermatozoa will be ejaculated after the thrusting has ceased. It is most important to keep constant pressure back of the glans until sufficient semen is collected. If the insemination is not to be made directly from stud to bitch, the semen may be collected in a test tube so that it can be more easily handled for the purposes of extending and shipping. Collection may be made directly into the test tube by means of a glass funnel or through the use of an artificial vagina.

Harrop has indicated his preference for the artificial vagina in all semen collections (Figure 64). It is interesting to note, however, that Boucher et al. (1958), working on the evaluation of quality,



Figure 63. Collecting semen directly into a syringe by simple digital pressure posterior to the bulb of the penis.

found that the artificial vagina had some detrimental effect on the quality of the semen. He, therefore, prefers the use of the glass funnel.

Motility and morphological examination of the sperm is carried out as soon as the sample is collected. A drop of semen is placed on a glass slide and spread slightly. It then can be examined under the low-power microscope for the amount of activity and motility exhibited by the spermatozoa. Observations also may be made as to the amount of mass movement, although this has little clinical significance.

For morphological studies, a very simple and effective method of staining is to mix a drop of semen with a drop of India ink. The abnormalities in morphology seen in other mammal sperm are also seen in dog spermatozoa, but the total irregular forms should not exceed 20 per cent. If a dried smear is to be examined, it is well to clear the slide of mucus with some chlorine solution before staining. There are many ways of staining such specimens.

If a "live-dead" count is to be made, staining the specimen with



Figure 64 Collecting semen by means of an artificial vagina

eosin B and nigrosin is very effective. The eosin will not penetrate the cell membrane in the case of living sperm, and dead sperm are stained a light pink. The nigrosin lays down a background against which the living sperm may be seen. There are many modifications of this method.

#### INSEMINATION PROCEDURES

After collection, the only additional equipment needed for insemination is a sterile tube (see Figure 62). An ordinary 1 ml glass pipette may be used for this purpose, or one may procure plastic insemination tubes which are sterile-wrapped by the manufacturer. A common size is 6 mm (diameter) by 1 cc (capacity). These plastic tubes cannot be autoclaved. In order that all equipment may be sterile and ready for use at any time, the glass pipette, syringe, and adapter can be wrapped in a muslin cover and autoclaved. Boiling is equally effective but has the disadvantage of consuming time when the insemination is being performed.

After the semen has been collected and examined, the bitch is

placed on a table of convenient height and held by an attendant. Most bitches will stand quietly to be inseminated, even though they may resist normal mating. An occasional bitch may be troublesome and in such instances it is a safe practice to use a short acting anesthetic while performing the operation. The genitalia are next cleansed with a mild soap and warm water and then thoroughly dried.

At this point one may insert a vaginal speculum and with a light locate the cervix. The inseminating tube is then placed directly through the cervical os. Most experienced operators, however, insert the tube without the aid of speculum and light. In this case the tube is inserted between the labia of the vulva and pushed in a vertical direction until it has passed the vestibule. The tube is then directed horizontally and passed through the cervix (Figure 65). The syringe is then connected to the tube by means of the adapter, and the semen is injected directly into the uterus (Figure 66). Some follow the practice of elevating the rear parts of the bitch following insemination. This may be of some benefit, but it seems logical to



Figure 65 Inserting the inseminating tube through the cervix of the bitch.

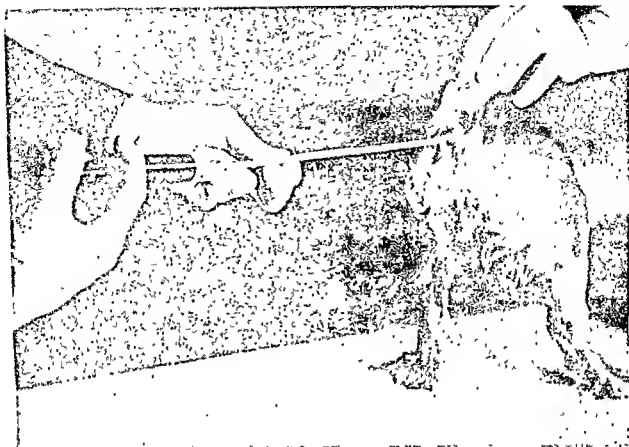


Figure 66. Injecting the semen under very slight pressure.

assume that if the semen is deposited in the uterus, elevation of the pelvic region is of little consequence.

#### FUTURE INVESTIGATIONS

Because of the limited life of the sperm in dog semen, widespread geographical distribution of the semen from a particular stud cannot be made without the assistance of some suitable extender. It has not been altogether possible in the light of the fragility of dog sperm to use all the proved buffers and extenders with the same degree of success as with bull semen. It seems quite possible that in the near future we shall be able to find ways of preserving dog semen so that it can be used at times and in places when and where a preferred stud is unavailable. Harrop (1954a) has recorded the first instance of a pregnancy following the use of preserved semen that had been shipped by bus and rail for a short distance. The author has recorded a successful pregnancy following the transatlantic shipment of preserved semen, collected and extended by



Figure 67 Beagle bitch with purebred puppies. These are the result of a transplantation of semen from a Beagle stud in London

Harrop and inseminated by Leonard (Leonard 1956) (Figure 67). This work has stimulated other researchers to investigate the field of dog insemination with the result that new extenders are being found and semen quality standards are now being set up.

The possibility of freezing dog semen has not been investigated, probably because this type of preservation might prove to be rather impractical in the case of the dog. This problem along with others connected with artificial insemination in the dog is now being investigated by workers who have recently become interested in the canine phase of artificial insemination.

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## Better Livestock Through Inheritance

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JOHN W. BARTLETT

Better livestock are the result of selection on the part of farsighted breeders. It is another way of saying that those men who have been successful in improving the heredity of their animals have decided which males and females shall be allowed to reproduce and which ones shall be discarded. Artificial insemination is being recognized because it permits many farmers to have the benefit of selected sires. Today we are mating millions of dairy cows to sires of known transmitting inheritance. Artificial insemination is a method which results in mass improvement in one generation. How far can this improvement be continued? Farmers have become conscious of the possibilities of selective breeding and they expect each generation to be greater producers than the parental one.

It has been said that we had just as great animals in our herds and flocks fifty years ago as today. That may have been true. We can now testify to the fact that artificial breeding to some of our great sires has given us many more great animals today than five decades past. We have demonstrated in the past ten to twelve years that the old Iowa experiment of increasing milk production over 100 per cent in two generations from scrub cows by improved dairy bulls can in a measure be applied to an industry. We shall continue this progress but how far can we go with improvement of our best



livestock? We do not have and no doubt never shall find, a way to create new determiners or genes for animal improvement

Artificial breeding of livestock by special matings is the best practical ally of the modern genetics of farm animals since the beginnings of animal husbandry. To have its greatest effect, however, artificial breeding should be a planned program. Its future really depends on our ability to select sires that will increase production, improve breed type and sire longevity with breeding efficiency.

There is an old adage: like begets like. It is a belief that in this statement lies the basis of all inheritance. True, each of our domestic animals reproduces its kind, but when we try to prove that parents and offspring are alike in detail we are in for a good many disillusion. Offspring do resemble their parents to a greater or lesser degree, because all potentialities of each newborn are dependent upon the continuity of the germ plasma of the previous generations. The characteristics, however, of any animal are determined by three factors. The first is inheritance. An animal can become great only through the inheritance it has received from its parents. The second controlling factor is environment, the external conditions which aid or hinder the animal's growth and development. Inheritance and environment working together constitute the third important factor. Although none of these factors can be omitted, inheritance or blood expresses the innate equipment to do things.

The study of inheritance is called genetics. A good definition of genetics is that it is a science which explains similarities and differences between parent and progeny.

#### LAWS GOVERN INHERITANCE \*

In 1900 three scientists working independently rediscovered certain principles that had been proved in the middle of the nineteenth century by an Austrian monk named Gregor Mendel. Their finding established the fact that many truly heritable characteristics of a plant or an animal are comparatively independent of one another and may be inherited independently. Where there are different characters in father and mother, such as horns and hornlessness, smooth coat and wrinkled coat in seeds, white color and black color in plumage of fowls, and long and short fur in rabbits, there is ob-

\* See list of definitions of genetic terms at end of chapter

vously a bringing together of the two traits in the resulting offspring. In the next or third generation, however, when these brothers and sisters are mated, the two distinct characters may be set apart again, thus showing that although perhaps one only was visible in the second generation, the factors which determine both were nevertheless present, and were moreover present in a separable condition. This is explained by our knowledge of the fact that those determiners which were present in parallel chromosomes in the egg of the mother and the sperm of the father and were separated by cell division have been passed on as distinct units. In other words, they can be separated in one generation and brought back together in another without losing their identity. Mendel had shown that this unitary nature of heritable characters always behaved similarly and that the old adage, 'there is nothing new under the sun,' is correct genetically.

Determiners, or genes, are always found in pairs—one member of each pair comes from each parent. Each gene has the power to maintain its identity from one generation to another, from father through a son to a grandson and even farther.

An offspring inherits one or the other determiner or particle, never both, from the pair its parent possessed. Thus a parent passes on to its progeny only half of its own inheritance.

An important qualification has been accepted by the modern geneticist, as we shall see later. It is that this independent assortment of characters is not always complete, since certain characters introduced together into a cross of two plants or animals tend to remain together in subsequent generations. They are members of a group which in heredity behaves independently of other groups. This is easily understood when we explain it by our knowledge of chromosomes which carry their genes in a linear order like towns on a railroad map.

*Segregation and Independent Assortment* So far we have seen that Mendel discovered two important principles or laws of inheritance: the first that traits of a plant or animal behave as a single unit in inheritance. Example: horns and hornlessness. An animal either has horns or is polled. The second law shows that genes, or determiners for traits, segregate from each other in the splitting of homologous or parallel chromosomes of the parents in preparation for fertilization of the egg by the sperm, disappear in the second generation and appear again in the third when the progeny, or brothers

and sisters, are mated Example A horned bull is bred to a polled cow Their calves have no horns, but in the next generation when these calves are mated, some of the progeny will have horns We shall call this 'segregation'

'Independent assortment' means that at the time division is going on in the egg and sperm before fertilization, the distribution of the chromosomes is random, forecasting a chance combination in the future If we consider a single pair of factors in each of two parents, such as black and red, we shall have four units, namely, two from each parent, and only one from each parent is used in the next generation Now these might come together in four possible combinations in the following generation Example The mother has determiners A for black and a for red, and the father A for black and a for red Then we may get combinations of AA, Aa, aA, and aa These different combinations are always formed in equal numbers but with no control

**Dominance.** One character may mask the other in the first generation in the crossing of two parents This means that when two individuals having different expressions of a unit character (polled x horned cattle) are bred together, only one of these characters will be seen in the first generation offspring (polled in the above example) This character is said to be dominant This will usually be the case if the two parents are pure for these characters The character that is masked or hidden in the first generation, or  $F_1$ , is said to be recessive (See Figure 68)

It should be kept in mind that these laws behave the same in either sex Thus the result will be the same in breeding a horned bull to a polled cow as in breeding a polled bull to a horned cow The crosses are said to be reciprocal Later on we shall see that there are some exceptions to these laws

As an example or two, we find first the case in which one unit character is not completely dominant over its paired gene If we cross a red with a white Shorthorn we get a roan Red is only partially dominant over white Another example is the behavior of unit characters which appear to work together rather than singly, as in the production of milk Milk production is accomplished by a combination of several heritable factors working as a group We cannot see the hidden characters of inheritance Their relation to each other or their position on the chromosomes of the female egg

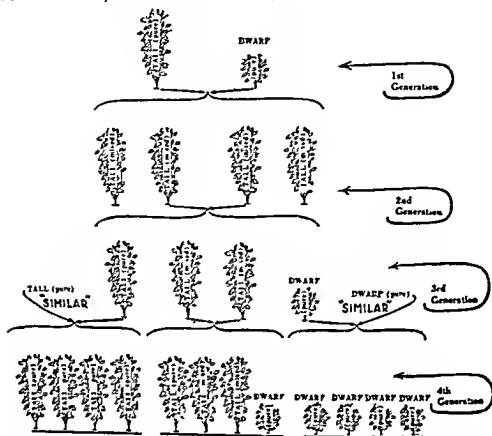


Figure 68 Mendel's breeding experiment. At the top are the tall parent and the dwarf parent that were first crossed to secure the first generation of all tall peas. The tall peas were mixed in their inheritance, although this could not be seen by looking at them, it is proved in the next generation, which consists of three tall and one dwarf. The dwarf in this third generation proved pure in its inheritance, for in further breeding work, nothing but dwarf peas resulted. One of the three tall peas in this generation also proved pure, however the other two proved mixed in their inheritance. The pure tall mated with pure tall always resulted in tall. The tall mixed carried on the proportion of three tall offspring to one dwarf, and so on, and on.

or the male sperm is called the genotype for the character. Their result, which we see in coat color, eye color, horn or hornless condition, and in milk, fat, wool, meat, etc., is called the phenotype for the character.

**The Backcross.** We can determine the genotype of individuals by the backcross, that is, if we wish to test an individual, we cross it with the pure recessive type. If the individual is hybrid for a character, the resulting progeny of the backcross will be one dominant and one recessive in equal numbers.

In attempting to check the genotype of livestock there must be

sufficient progeny to give reasonable assurance that the correct ratio is obtained. If, for example, we wished to check a Holstein or an Angus for a red factor, we would mate the animal with a recessive red such as the Guernsey or the Hereford. Unless we used several crosses in the same parents, we might not have assurance that the dominant parent was free of the recessive red.

**The Di-Hybrid Cross.** When we mate animals, we naturally deal with the inheritance of more than one character. That is, we deal for example with black color versus red and polled versus horned at the same time. Let us cross an Angus and a Hereford in which there are two pairs of genes that are unlike or heterozygous. All possible combinations can be represented, and the characters will assort independently in the eggs and the sperm. There are four possible genes from each parent. The results will be in the same ratio, however, with nine black polled animals, three black horned, three red polled, and one red horned. One can work it out by a checkerboard if one wishes.

**Lacking of Dominance.** When we mate red and white Shorthorns, we get roan progeny. Thus the result of the gene action for red and for white appears in the same animal, yet in the  $F_2$  generation we get one red, two roan, and one white again.

If the breeder wishes to breed roan cattle, he must have red cows and white bulls, or vice versa.

The breeder should recognize at this time that he cannot tell by looking at an animal or a fowl what it will transmit. We know that it can transmit only what it has inherited from its parents, but we may not know what characters have been in the ancestors of the animals with which we are working.

We have in poultry a situation which is confusing until we understand that two pairs of factors influence only one characteristic instead of two. The condition in comb inheritance is the result of complementary factors. If we cross a Rose comb with a Pea comb, we get a Walnut comb. When these hybrids are mated, we get Walnut, Rose, Pea, and Single.

When Rose-combed and Pea-combed are crossed, the results are Walnut-combed; and crossing two Walnuts, we get nine Walnuts, three Rose, three Pea, and one Single, again a 9:3:3:1 ratio.

**Multiple Factor Inheritance.** So far our discussions have dealt with the inheritance of more or less easily distinguishable characters. In brief, the progeny tend to resemble one or more of the ancestors in

some particular way, such as color of hair, presence or absence of horns, and so on

This is explained by the fact that one or a few sets of allelic factors are responsible for the characters manifested. Even in the case of the roan Shorthorn the result is due to the admixture of two simple colors.

If we study the inheritance of milk, size, speed, and fertility, it cannot be explained so simply, yet rules of inheritance are still at work. If we take size in dairy cattle as an illustration, no precise divisions in groups can be made. True, we can easily distinguish between a 1,500 lb cow and a 900 lb cow, but we cannot say that a 1,200 lb cow is large or small, and in a herd we shall find cows weighing from 1,200 to 1,800 lbs. The reason for so many intermediate forms being produced is that size in this case is governed by several sets of gene pairs probably found on several different chromosomes.

For example, dominance for any single factor does not play an important part in size. The question of whether an animal is large, medium, or small is determined by the number of genes for large size present in proportion to the number for small size. Thus we can see that the effects of these sets of genes, which are responsible for a particular character, are not only equal but add up to some result. The more genes for large size, high production, or speed present, the greater size, production, or speed we find in the animal. On the basis of multiple factor crosses we sometimes get  $F_2$  animals more extreme than the grandparents. This is because each parent contributes some effective inheritance not supplied by the other parent. Many multiple series are known in plants and animals. Quite recently it has been discovered that there are heritable antigens in the blood of cattle and birds. Already at least two score of these antigens have been traced in cattle, and we are now using them to prove parentage in the case of disputed matings in artificial breeding. The day may be near at hand when all purebred dairy sires will have to be blood typed before the breed associations will record their progeny.

**Sex Linked Inheritance** In all examples of inheritance thus far shown, no reference has been made to sex. It has made no difference in which parent the set of characters was placed. That is, it could be from the male or from the female. In sex linked inheritance we

find that the genes determining certain characteristics will be located in the chromosomes that determine the sex of the individual.

The common fruit fly has been used in the laboratory for a study of many of our facts on breeding work. It has four pairs of chromosomes. In the female these are all evenly paired, two pairs being large bent rods, one pair very small and oval-shaped, and one pair long and rod-shaped. The last pair are the sex or X chromosomes. In the male the makeup of the first three pairs is similar to that in the female, but in the fourth pair we find one sex chromosome a duplicate of that in the female and the other somewhat smaller with a bent portion at one end. This is called the Y chromosome. Whenever an X and Y appear together, the sex has to be male.

In the breeding work with the fruit fly, which normally has red eyes, a white-eyed mutant male was discovered. This male was saved and mated to red-eyed females, and all the progeny were red-eyed. In the next generation, however, the expected three to one ratio of color was apparent, but all the white-eyed individuals were males. This phenomenon was explained by finally locating the genes for white eyes in the X chromosome. When a red-eyed female was mated with a white-eyed male, the flies would have red eyes, but the female would also carry in one chromosome the factor for the white eye. When mated to a red-eyed male, she in turn would yield two red-eyed females, one red-eyed male, and one white-eyed male.

The female produces only one type of sex chromosome, whereas the male produces two types, one an X and one a Y. The sex depends on which type happens to come from the male. All characteristics that are determined by genes carried in the male X chromosome will therefore show this peculiar type of sex-linked inheritance. Many such characteristics have been located on the chromosome map.

There are a number of known sex-linked factors in poultry. If the barred rock cock is mated to a black or any other non-barred breed, the offspring, both male and female, are barred. When the  $F_1$  hybrids are mated, producing an  $F_2$  generation, the results are three barred individuals, two males and one female, and one black or non-barred female. When the reciprocal cross is made, namely, black male on barred females, the chicks in the  $F_1$  are produced as barred males and not barred or black females. When these hybrids are mated, the resulting generation consists of one-half barred and

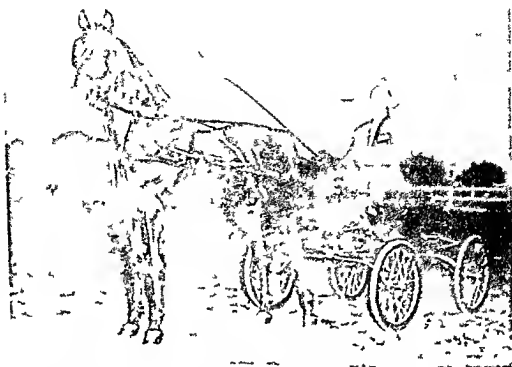


Figure 69 Dyberry Robin a fine example of a young Morgan horse is the product of 804 crosses back to the original Justin Morgan. Raised and trained by his owner Elmer N. Searls of Madison, New Jersey, he placed second in the three-year-old stallion fine harness driving class of the 1958 National Morgan Horse Show. At the present time registered Morgan horses are all products of natural mating.

one half black males, and one half barred and one half black females.

**Sex Influence Inheritance.** We must not confuse this type of inheritance with sex linkage. In this case the influence does not come from the X chromosome but from the other chromosomes, called autosomes. They are responsible for the production of the so called ductless glands which secrete hormones. The pituitary, apparently the most important of the ductless glands, lies in a bony socket at the base of the brain. It is known to secrete a dozen or more hormones, some of which incite and regulate sexual manifestations as well as lactation. The female has ovaries, and the male has testes. These glands also have an endocrine function which is manifested by differences in the appearance of the female and male. In sheep some breeds have horns only in the male. Some breeds are hornless in both sexes. In Ayrshire cattle there is found a type of inheritance



which produces mahogany and white in the male and red and white in the female. In poultry the difference in feathering between males and females is due to testicular and ovarian hormones.

Another type known as sex-limited inheritance is the result of sex hormones. The crest in the bulls, the masculinity in the stallion, and bright plumage in the cockerel are all examples of this hormone peculiar to the male.

*Inheritance of Lethal and Sublethal Traits.* So far in our study of inheritance we have dealt only with those units or genes which are responsible for producing some observable character. B, for black color in the Angus cow, will always produce for us a black calf when it appears. There are, however, genes which, when they come together in a homozygous state, produce such a drastic action that they cause the death of the individual. These are called lethal genes. They may exert their killing effect in prenatal life or at birth of the individual. In a broader sense a lethal gene is one which leads to the premature death of the individual, whether it be in utero, at birth, or after birth. We often hear the latter two spoken of as sublethal or semilethal. Some lethals have been found which cause death of the gametes, others during the formation or early life of the embryo, and others at various stages of uterine individual and postnatal life. One of the best-known lethals in animals is found in the Irish Dexter breed of cattle. The result is the so-called bulldog calf. In the heterozygous condition the Dexter is a short-legged, broad-headed individual native to Ireland. When it is interbred, it produces a ratio of one-half of the Dexter type and one-fourth of the Irish Kerry type. The pure, or homozygous Dexter, resembles a Scottie dog and dies in utero. The skull is vaulted, the lip is cleft, and the lower jaw and the tongue protrude. Such calves are usually aborted between the third and the eighth month. Delivery at full time is difficult because of the huge head.

In England the practice has been adopted of breeding Dexter to Kerry, getting one Dexter and one Kerry, thus avoiding the chance of getting the bulldog lethal.

It is most important in selecting sires for artificial breeding that we be assured that each sire is free of all lethal and/or sublethal characters. Recently the various breed associations have requested their membership to report any sire whose progeny show any one of the serious characters. No doubt a plan for testing all sires to

be used in breeding some day will be required. Many practical breeders now breed their sires back to their own daughters to test their inheritance before using them extensively in a breeding program.

The lethals and sublethals that are common in domestic animals, as listed by Eaton (1937), are as follows, with indications of whether they are recessive or dominant.

### LETHALS AND SUBLETHALS IN DOMESTIC ANIMALS

#### *Horses*

Atresia coli—closure of intestine		R
Abnormal sex ratio—55 males 90 females	Sex linked	R
Lethal white—low fertility—lethal or sterility factors or both		?
Stiff forelegs	Probably	R

#### *Cattle*

Achondroplasia—(Bulldog) short legs and head, hernia, die and aborted fourth month	D
Achondroplasia—(Bulldog) short head cleft palate deformed jaws die soon	R
Acroteriasis congenita—amputated—appendages short or absent	R
Agnathia—very short lower jaw (sex limited to male?)	R
Ankylosis—ossification of joints	R
Congenital dropsy—water in tissues and cavities	R
Congenital ichthyosis—scaly, cracked skin	R
Epitheliogenesis imperfecta—imperfect skin, partly hairless—septicemia	R
Fetal resorption	?
Hernia cerebri—failure of frontal bones to fuse	?
Impacted molars—short jaw, defective teeth—die within week	?
Lameness in hind limbs—calves unable to stand	R
Muscle contracture—head and legs drawn up—joints stiff	R
Mummification—short, stiff neck, prominent joints—usually die as eight month fetus	R
Short limbs—limbs short, hoofs undeveloped	R
Short spine—ribs and vertebrae fused, back bent down	R
Dwarfism—not distinguished until one year of age	R
Blindness—small eyes and cataracts	R
Cross eye—detectable at six months to one year	R

Notched ears—ears are very short	Semi	D
Syndactylism—only one toe on one of four affected feet		R
Flexed pastern—feet turned back		R

### Sheep

Amputated—no claws on feet		?
Earless and cleft palate		R
Lethal gray—in Turkanas and Karakuls		R
Muscle contracture—usually stillborn		R
Paralysis—hind parts paralyzed—live few days		R
Skeletal defects—large head, short upper jaw, rigid fetlocks		R

### Swine

Atresia ani—closed colon		?
Catlin mark—parietal or frontal bones not fused		R
Cleft palate—young unable to nurse		R
Excessive fatness—young die at 40 to 80 kg.		R
Fetal mortality		R
Hydrocephalus—water outside brain in subachnoid spaces		R
Hypotrichosis—hairless (lack of iodine)		?
Legless—(Shoulder blades and pelvic bones but not limb buds or leg bones)		R
Lobed ear—usually also cleft palate and deformed hind legs	Probably	R
Muscle contracture—thick, stiff legs		R
Paralysis—hind parts paralyzed		R

**Linkage.** We have seen in the foregoing descriptions of inheritance that several types of behavior exist in regard to the manner in which characters are passed from generation to generation. Generally, we have seen that characters are dependent on one pair of genes. These genes sometimes come from the sex chromosome and sometimes from the autosomes. Several cases of dominant and recessive lethals have been mentioned. Still another type of manifestation appeared in which two or more genes showed their effect in a multiple series.

Further summary reveals that sometimes two pairs of genes act together to give one result, and again we saw examples of lack of dominance, as in the roan Shorthorn. In several of these types of inheritance the 3:1 or the 9:3:3:1 ratio had exceptions.

In 1910 another peculiar exception to Mendelian inheritance was described by Morgan (1931) who was at that time at Columbia University. The new exception was called *linkage*. It has been explained that each chromosome consists of hundreds of genes situated in linear order. Dr. Morgan and his co-workers put forth the theory that when two genes are close together in a chromosome there is a tendency for some of them to pass from generation to generation together and for their end results to be inherited together. Most farm animals have a large number of chromosomes and two genes selected at random are likely to come from separate chromosomes and act independently. If we take some character like size, speed or production it is affected by several pairs of genes and there is a strong possibility that some linkage exists among certain of them. Because of a tendency, however, for certain sections of chromosomes to cross over to others during division the benefit of linkage is equally offset and a breeder cannot get the effect of one gene by selecting another close to it.

**Mutations** Mutations are chemical rearrangements of the material of which genes are made. The result of a mutation in a gene is evidenced by a change in the inheritance normally caused by that gene. It may behave as a dominant or a recessive. Mutations are rare occurring perhaps once in every 100,000 cell generations. We do not know much about the causes of mutations. The first mutation ever discovered was white eye color among red eye fruit flies in the biological laboratory of a large university. Among farm animals the best illustration of a mutation is polledness or lack of horns in certain cattle. Most mutations are never recognized. They are rarely beneficial, often harmful. Because of their rarity a breeder can never hope to improve his livestock by finding a mutation. Should he ever be so fortunate as to discover one it is an easy Mendelian problem to establish it in a family.

#### GENETICS AND BETTER LIVESTOCK BREEDING

To the average breeder of livestock the foregoing facts may tend to be confusing and he will ask "so what?" A knowledge of basic principles and an assurance that they apply to all types of livestock inheritance should give the breeder confidence in trying to improve his flocks and herds. The principles laid down have never yet been contradicted or disproved. The breeder must recognize that

most of the principles are general and must be applied in a general way. They are given only as a guide, and they do serve to explain why things happen as they do in the mating of our various types of livestock.

*Variation as a Tool.* Perhaps the previous statement of how inheritance works will serve to explain why no two calves, no two chickens, pigs, horses, or even humans, are just exactly alike. It is very well said that variation is both the hope and the despair of the breeder. Because cows vary in size, color, milk production, fat percentage, and longevity, there is always the opportunity for improvement. If we take a census of all livestock, we recognize that variation is really almost unlimited. We find, for example, that the milk of the Holstein cow averages, for the breed approximately 37 per cent butterfat, but the variation extends all the way from below 3 per cent to above 45 per cent. The New Jersey Holstein Breeding Experiment has been developed since 1932 around a selected group of 30 females, all of which tested from 38 to 44 per cent. Since then several scores of cows descended from the original group as a result of matings with sires of known high butterfat inheritance. The average of all these cows is 40 per cent of butterfat, and the milk production of the present group is above that of the foundation animals.

Variation is a tool then for the improvement of livestock. It provides the breeder with the genes to fashion higher production, better udders, stronger legs, or straighter rumps. The alert breeder is always looking for the exception in hope that it may give him the desirable change that will be of economic importance to the livestock industry. He does not create anything new. He merely recognizes new combinations of factors and attempts to hold them by certain matings. He discards animals with an inheritance that he does not want, and he discards them. He puts together what he likes with the hope that he can breed future progeny with the good points of the selected parents.

*Controlling Inheritance.* There are only a few practices that a breeder can use to change the inherent makeup of his livestock. He can decide which ones will be allowed to become parents and which ones he will discard. Also he has his choice with those he selects as to whether he will breed together those which are alike or those which are unlike. In brief, he can breed likes to hold certain characteristics, or he can mate unlikes with the hope of changing certain characteristics.



Figure 70. Marlu Milady and her daughter Marlu Milady's Fashion, of Marlu Farm, Lincroft, New Jersey. Marlu Milady has a record of 25,293 lbs. of milk and 1,210 lbs. of butterfat.

The rapidity with which changes are brought about depends upon the frequency of the gene which influences the change in the new combination. For example, if we were selecting against a red factor in Holsteins or Angus, our success would depend on the frequency of the gene for red among the animals selected to be parents.

In selecting animals for breed changes, the breeder considers not only how their superior characteristics outweigh the inferior traits but also how the animals selected compare with other animals available to him. Practically speaking, the breeder selects his animals for breed improvement on the basis of net merit. The rate at which a breeder can change his herd or flock depends upon how far he can afford to go in discarding the undesirable animals. The difference between the average for certain factors in his herd or flock and the average of those he saves for breeders is called the selection differential. Therefore, in order to avoid mistakes the breeder should set up goals, systematic scoring with yardsticks of individual merit.

*Use of the Yardstick.* For the purpose of studying the improvement a breeder is making in his animals, there are various analyses that

should be made regularly. Many colleges of agriculture are now making available to breeders a herd analysis service for production. Charts are so organized as to permit an examination of cow families, or the descendants of certain cows through three or more generations. The daughters and granddaughters of an individual cow are compared to their half sisters by the same sire. A comparison on this basis gives a breeder the opportunity to select certain of his superior female lines for concentrated breeding, and to discard those that do not measure up. This type of analysis may include birth weight, growth weight, height, heart girth, and health, also conception rate, calf mortality, and longevity. It can be used for all classes or breeds of livestock and should be a part of the annual inventory to determine what variations for good or bad are taking place. It reveals the weaknesses that exist in the herd or flock and immediately gives the breeder an idea of what he must keep in mind in selecting future males for mating.

Type classification reveals strong and weak points of conformation. Through herd classification, the breeder has an opportunity right on his own farm to rate his own cattle with the ideal type and to compare them with the average of the breed. Classification permits the breeder to recognize animals of superior type and to eliminate those that are less desirable.

#### CLASSIFICATION STANDARDS \*

- A. *Excellent.* Animals must be scored according to the official scale of points and found to score 90 points or more before being officially designated Excellent.
- B. *Very Good.* Animals which in the opinion of the judge would score approximately 85 and less than 90.
- C. *Good Plus.* Animals which in the opinion of the judge would score approximately 80 and less than 85.
- D. *Good.* Animals which in the opinion of the judge would score 75 and less than 80.
- E. *Fair.* Animals which in the opinion of the judge would score approximately 70 and less than 75.
- F. *Poor.* Animals which in the opinion of the judge would score less than 70.

\* Score cards for all forms of livestock may be secured from the Breed Association Offices.

Using the above standard Holstein cows are classified on the basis of

- |                           |                  |
|---------------------------|------------------|
| 1 Detailed classification | 5 Mammary system |
| 2 General appearance      | 6 Fore udder     |
| 3 Dairy character         | 7 Rear udder     |
| 4 Body capacity           | 8 Feet and legs  |
| 9 Rump                    |                  |

### DEFINITIONS \*

**Chromosome** One of the rod like or rounded bodies into which the chromatin of a nucleus is resolved at the time of cell division. The cellular elements regarded as the carriers of hereditary characteristics.

**Unit characters** Any characters of any organism that behave as a unit in inheritance—plant size in peas, horns in cattle and so on.

**Genes** The units of inheritance that probably react together and with the cytoplasm and these two with the environment to make patent the organism's latent potentialities.

**Allele** Mendelian characters are inherited in alternative pairs (or series). These alternative forms of a gene which are located at the same point on each one of a pair of chromosomes are called alleles for example horns (recessive) hornlessness (dominant).

**Dominant** A character possessed by one of the parents of a hybrid, which is manifested in the hybrid to the apparent exclusion of the contrasted character from the other parent (the recessive).

**Hybrid** The individual that arises from crossing parents which are pure for certain different characteristics.

**Phenotype** The expressed character (or sum of all the characters) of an organism.

**Genotype** What an organism actually is as determined by its germ plasm.

**Heterozygote** An organism to which its two parents have contributed unlike genes with respect to any given allelic pair governing contrasted characters and which in turn produces two kinds of germ cells with respect to the character (Heterozygous adjective).

**Gamete** A reproductive cell of either sex, a sperm or egg.

**Heredity** The resemblance derived from the ancestry among organisms related by descent.

**Variation** In biology the occurrence of differences among the individuals of the same species or variety.

\* Definitions taken from Rice 1951



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## Systems of Breeding

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JOHN W BARTLETT

A system of breeding is in reality a method of controlling inheritance. If a system fails, we may say that it is not the fault of the system but of our selection of animals with a poor inheritance. Improvement in livestock is under our control only insofar as we are able to detect, and to raise progeny only from livestock which carry, those characters we want. Rice says in *Breeding and Improvement of Farm Animals* that every breed has a certain number of good genes and a certain number of bad ones. An individual breeder, then, can hope for progress only when he can increase the frequency of the desirable ones or can look for recombinations into favorable groupings.

There are four principles that every breeder must learn early if his work is to be a success. He must have an idea of what he wants to accomplish and adhere strictly to it. He must have a clear idea of the history of the animals with which he is going to work and must recognize their strong and weak points. He must know how far he may go in creating variations and still get back to his original goal. He must keep in mind that to be successful his program should be economically sound.

Every breeder should recognize that his own animals have a storehouse of heritable characters. Even within his own herd or flock, selection may be able to give him many of the results which would

improve his average. Most breeders, however, attempt to make changes in their herds and flocks by the introduction of males from other breeders. In the Holstein breed the range of butterfat percentage has been shown to be over 1 per cent without any apparent change occurring in milk production. Certainly those animals in the high brackets afford an opportunity for selection. Not many years ago the average hog was short-legged and deep-bodied. It was a so-called lard type. Today we have completely changed the accepted type of hog to a longer-legged, strong-backed animal of the bacon type, with extra large hams of greatly increased commercial value. All this was done by systems of breeding and intelligent selection.

#### HOW CAN A BREEDER CHANGE HIS ANIMALS?

All who knew him will agree that James E. Dodge\* was a master breeder of horses, cattle, and hogs. He once said that if a man could see the faults in his animals, it was an easy matter to change them. If the faults can be seen, he has the power to determine which animals shall have progeny and which ones shall be discarded. This is selection and was used by breeders long before we knew anything about the laws of inheritance. There are several methods of selection, and few breeders agree on what is the correct one. When we analyze the various methods, it is clear that selection is based on individual merit, pedigree, progeny, or a combination of all three. Many breeders cannot explain their successes but intuitively have mated their animals on the basis of similarity or difference, whether in individuality, progeny, or pedigree. In brief, then, we like to perpetuate desirable characteristics, or we mate unlikes to change an undesirable characteristic to a more favorable one. If our animals are related, we practice some degree of close breeding; the more closely related, the greater the intensity. If no blood relationship exists in our matings, we are practicing outbreeding.

As an illustration, there was a fad only a few years ago for white Holsteins, and breeders always bred light to light to maintain this fad. On the other hand, Carstons Bridesmaid, a very beautiful Ayrshire cow, was imported to this country from Scotland principally to produce a son whose daughters might have the perfect udder of

\* Manager of Emmadine Guernsey Farm, Hopewell Junction, New York.

their paternal grandam. A straight-legged sire is often selected with the hope of correcting an inherited weakness in the legs of the daughters of another sire.

### GRADING

Grading is the practice of using purebred sires on native or inferior females. The system began after it had been demonstrated that sires with a long line of pure breeding and selection would improve milk production, wool length, or other qualities when mated to low grade females. It has recently been shown in the Holstein Breeding Experiment at the New Jersey Experiment Station that sires with a few generations of inbreeding for high butterfat percentage surpass outbred sires from a high testing dam in passing on the high butterfat test factor when mated to grade cows or unrelated purebreds.

The first generation resulting from crossing highly inbred, selected sires on inferior females usually results in a very noticeable change for the better. The result is that the progeny carry 50 per cent of the blood of the better sires. The following generations will show less improvement as their genetic makeup becomes more and more like the superior purebred parent, and the percentage of change in each generation becomes less and less. As an example, the second generation carries 75 per cent pure blood, the third 87.5 per cent, and so on until, after five or six top crosses, the amount of pure blood approaches 100 per cent. On this basis the original cow's influence becomes less and less, but to a smaller degree in each generation. For those dairymen who subscribe to the plan of breeding to superior proved sires in artificial breeding associations, this will be particularly true, and the more highly improved their cows may be, the less rapid will be the change for higher production as compared with a neighbor who may have a low producing herd. One of the principal functions of an artificial breeding organization is to furnish the commercial farmer with superior seed stock. The commercial producer is therefore grading up with a superior dose of good inheritance upon which he can depend to improve his animals. The cost of such a program is without doubt quite low to the individual who cannot afford to own a superior sire. The breeder of purebreds has long recognized that grading is sound, because it offers a market for his meritorious sires.

## CROSSBREEDING

Crossbreeding is the mating of purebred animals belonging to different breeds. It has been done in the past few years by crossing the high milking breeds of dairy cattle with the high butterfat testing in an attempt to get both high production and high butterfat content.

It has been more generally used in the past with meat animals, because it usually brings about an increase in size and vigor. Crossbreeding, as the term implies, brings together animals of different genetic makeup and results in many kinds of variations. Generally, it is unwise to breed the crossbreds to either parent purebred strain, because immediately the recessive characters appear. Some of the unfavorable ones of each parental strain might appear in the succeeding generation. Robertson (1949), the English geneticist, has shown that in one recent experiment with dairy cattle, crossbreeding seems to have had a heterosis effect of 15 to 25 per cent when the production of purebreds and crossbreds from the same dams were compared. Therefore it can be said that crossbreeding has its advantages as well as disadvantages.

During the past twenty-five years a number of crossbreeding experiments have been conducted. At the Maryland Experiment Station it was shown in 1941 that crossbred Plymouth Rock poultry from the same sires were superior to the purebreds in feed utilization in the production of broilers.

Crossbreeding between different breeds of swine has long shown some advantages. At the Illinois Station a double litter of pigs from a purebred Duroe boar and a purebred Poland China boar on a Duroe sow contained six Duroe Jerseys and four crossbreds, which were spotted red and black. The birth weight of the purebreds was 3.23 lb., and the cross bred averaged 3.75 lb. The females were weighed at six months of age after being raised under identical conditions. Two remaining purebred females averaged 185.5 lb. each, and the female crossbreds weighed 228 lb. This is a striking example of the advantage of cross breeding.

On several occasions grand champion steers at the International Livestock Show have been crosses between Herefords and Angus or Shorthorns. Toggenburg goats crossed with Nubians appear to make

superior milk producers, and their kids have a decided advantage as meat. Sheep breeders have for a long time practiced crossbreeding. Hampshire Down rams on Kerry Hill ewes have been most successful in giving thickset, well fleshed, and hardy lambs. Suffolk rams have been in great demand in England for outcrossing with ewes of other breeds to produce fat lambs which put on weight rapidly. Everyone knows of the sturdy, tough qualities of the mule. Crossbreeding in dairy cattle has been rather limited because dairy cows, to be profitable, should be kept in production for several years. The second cross of a crossbred carries 75 per cent of one parent, therefore the resulting animal is likely to show more of the qualities of one breed than the other, sometimes to a decided disadvantage.

In commercial herds some dairy men use a Brown Swiss sire as a top cross, and the resulting calves command a premium price in the veal market. In some artificial breeding organizations, a beef bull is used on commercial herds for the same purpose. With the use of the Angus sire upon dairy first-calf heifers, the progeny are often smaller at birth than when a dairy bull is used.

It may be concluded that crossbreeding is a successful program in producing meat animals of superior quality. It should be used only one generation. Therefore it is necessary to carry purebred sires of two breeds in order to have parent stock. The artificial breeding program takes care of this and offers sires of known superiority for crossbreeding opportunities.

#### OUTCROSSING

Outcrossing is the mating of animals of the same breed but with no relationship for at least the first five generations. An outcross is often called a cold blood cross. It is usually the use of a male on a closely bred group of females or on a single female to introduce some desirable inheritance or to overcome some factor which seems to have developed in a closely bred family. An outcross is sometimes used to continue or retain a heterozygous type, where close breeding would result in undesirable factors. For this reason some breeders prefer to follow one good hull with another not very closely related

## CLOSE BREEDING

Close breeding means the mating of relatives within a family.

The mating of animals which have a common ancestor in the first four or five generations is generally classed as close breeding. For the purpose of this chapter, close breeding will be divided into two categories, namely, inbreeding and line breeding. Inbreeding includes matings of full brother and sister, father to daughter, and mother to son. Line breeding includes half brother and sister, first cousins, and other animals more distantly related but with a common ancestor.

Close breeding is a means of purification of stock, for it brings to light weaknesses which may remain hidden for generations. In brief, if we may appreciate its possibilities, it becomes a tool for ridding a family of certain undesirable characteristics and of purifying it for those we would favor. It does not create anything new, but with selection it permits pairs of recessive genes to become fixed. Helen Dean King of Philadelphia has probably done more to illustrate the possibilities of close breeding than any other breeder. She selected a pair of large white rats. From the offspring of this pair she selected a full brother and sister with one thought in mind, to maintain size and vigor. For seven generations she closebred and selected the improved individuals until she had apparently secured a pure inheritance. So she has continued to breed this family for 80 generations without any bad results. If breeders may take a lesson from Dr. King, it does not seem impossible to develop superior strains of domestic animals by close breeding.

The great role of inbreeding should be the elimination of the bad traits and the doubling up on the good ones. It should be obvious that if closebred animals become so weak that they cannot live, it is well to rid the breed of them.

## INCROSSING OR LINE CROSSING

The development and crossing of inbred lines is somewhat of a new philosophy in livestock breeding. Experiments with plants (hybrid corn) and swine have resulted in progeny superior to the better parent. At the California Experiment Station it was demon-

strated that crossing an inbred sire on unrelated inbred females resulted in heterosis with dairy cattle as well as with hogs. Results obtained among dairymen in that state in the use of inbred dairy sires from different lines on closely bred females indicate a practical value from this system of breeding.

#### IS INBREEDING A SAFE METHOD?

If we really understand inbreeding, we quickly recognize that both good and harm may come from its practices. Inbreeding does not create, neither does it destroy. All that any breeding system does is to recombine the inheritance factors or genes that already have been inherited from a previous generation into recombinations which will be exhibited by the new generation. Each present individual is merely a progenitor carrying in himself or herself the combination received from the two immediate parents. If we have a preponderance of good factors in our stock to begin with, then in breeding and selection will improve the future generations. The writer knows several breeders who breed the first few daughters of a sire back to him in order to find out if his genetic makeup is good or bad.

At the University of California, Mead, Gregory, and Regan (1949) report a lethal which has appeared in a herd of inbred Jerseys. The sublethal type of achondroplasia differed from the Telmark type in that it was a milder and more variable manifestation. The examination of the pedigrees of 11 affected animals showed the defect to be inherited as a simple autosomal recessive.

The appearance of this lethal might have remained unnoticed had inbreeding not been practiced. It was not the inbreeding that caused the lethal but inbreeding was a method which revealed that it existed in this family of Jerseys.

Winters and associates at the University of Minnesota have recently conducted some extensive inbreeding work with hogs. The three items that received the most attention in the development of an inbred line of swine were fertility, the rate of gain from weaning to 200 lb. and the economy of gain. The experiment raised the coefficient of inbreeding to 0.24 and showed that the poor performance and deterioration generally assumed to accompany the inbreeding



of an  $F_1$  generation did not occur. Also, this experiment points the way to a new method of animal improvement.

It has been shown in the New Jersey Holstein breeding studies that lethals and deformities will appear in some families and that production and size will go down in others. In other families good results were attained. Inbreeding, plus rigid selection, was successful. But it is perhaps safer to line breed to some outstanding female or male than to take the risk of inbreeding.

In the breeding of poultry it is reported by Jull (1952) that many recessive genes are less desirable than dominant ones, and inbreeding concentrates these undesirable recessives which have been hidden by the desirable dominants. "Inbreeding may be practiced to some extent with rigidly selected stock, and in this way the undesirable characters can be eliminated.

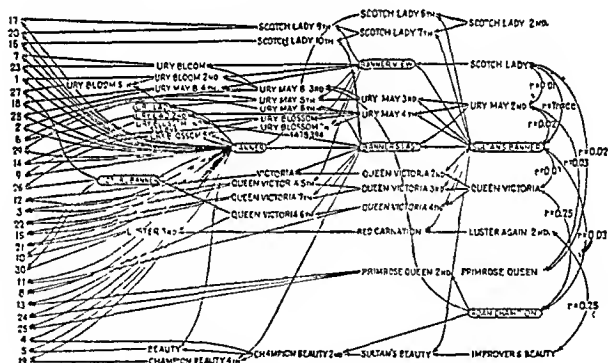


Figure 71. Five generations of inbreeding in Beef Shorthorns. Pedigree of the entire young herd late in 1932, complete back to the time of Sulton's Bonner, and with coefficients of relationship ( $r$ ) based on the ancestors alive in or near 1900. Each animal is shown but once. If it has more than one son or daughter, an additional arrow indicates that selection had not yet had much opportunity to thin out the young herd, but with that inevitable culling, many of the lines from Bonner will have disappeared in the herd ten or fifteen years later, even though the present plan was continued. Bonner may not appear much more important than Bonner View or Bonner's last. In any case, he is not apt to be more important than those two combined (Lush, 1934).

Line breeding eliminates the danger of mating full sisters to full brothers, which might bring out undesirable characters. Line breeding requires the purchase of one good male and rigid selection of hens for breeding stock."

He says crossbred stock may be superior in many ways because of hybrid vigor. "The progeny of crossbred stock are of less value for breeding than the parents, therefore it would be necessary to maintain two strains of purebred stock for crossbreeding—a handicap for the average breeder."

Lush (1934) has reported a herd of Beef Shorthorns bred for 20 years without new blood being introduced. A composite pedigree of this herd is shown in Figure 71.

Probably one of the greatest demonstrations in breeding has been that of the development of the Glenburnie family of Guernseys by James E. Russell (1936). From 1906, when he purchased King Masher 11084 until his death he practiced line breeding and some inbreeding in three strains of foundation animals. His greatest bull cost \$75 and he never paid a high price for any male or female. From this herd, however, has come foundation stock for a great many new breeders. Sales have shown that the public has confidence in a long line of breeding. To quote Dr. Russell in 1936: "I can say unqualifiedly that there has been no loss of constitution and vigor. King Masher's daughters ranged in weight from 1,150 to 1,300 lb. Twenty-five years later the get of Dunwalke Lad 113342 matched them pound for pound. I note that type improves with each generation of inbreeding with some loss in size. In productivity my best claim to success is that I have increased the average production of the herd." An example of one of many great Glenburnie cows is Glenburnie Golden, which produced 10,947 lb of milk and 644.9 lb of butterfat as a Junior two-year-old. She is the result of close breeding and selection (Figure 72).

The New Jersey Station used close breeding in an attempt to breed a family of Holsteins with a high butterfat test. Females with average butterfat tests of 3.8 to 4.2 per cent were mated with sires proved for high test. Progeny of these matings were crossed and sons and daughters saved for further close breeding. Conclusions indicate that the fat tests have increased twice as fast by close breeding as



Figure 72. Glenburnie Golden of four years of age, a product of thirty years of constructive breeding in the Guernsey herd of Dr. James E. Russell, Lawrenceville, New Jersey.

by mating high-testing cattle without attention to family relationships.

**The Coefficient of Inbreeding.** In the selection of sires to be used in artificial breeding it may become important to ascertain some quantitative measure of comparative matings. For this purpose a formula known as Wright's Coefficient of Inbreeding may be adopted. It is written as follows:

$$f_0 = \Sigma (1/2)^{n+n'+1} (1 + f_a)$$

This formula can be explained upon the appearance of common ancestors in the pedigree of both the sire and dam of an inbred or closebred animal. If we may call a common ancestor A, then the number of generations from sire  $n$  and dam  $n'$  are added together and increased by one (the animal in question is one generation from its parents), and this value is used as the exponent of one-half, one-half being the possible contribution of each parent. If the common ancestor A is inbred, his coefficient of inbreeding  $f_a$  is determined by the

TABLE XVIII

Individual	Common ancestors of sire and dam	$f_a$	$n$	$n'$	$\frac{1}{2}n + n' + 1_{\{1+f_a\}}$
A	H	0	2	1	0.0625
	G	0	1	1	0.1250

Coefficient of Inbreeding = 0.1875

TABLE XIX

Individual	Common ancestors of sire and dam	$f_a$	$n$	$n'$	$\frac{1}{2}n + n' + 1_{\{1+f_a\}}$
Y	A	.1875	0	1	$\frac{1}{2}^2 = .25 \times 1.875 = .20685$
	E	0	1	1	.1250
	G	0	2	2	.0312
	H	0	2	2	.0156

Coefficient of Inbreeding = 0.4687.

Wright also says that when we inbreed, we reduce the percentage of heterozygosis. If we recognize that each parent may transmit 50 per cent to its offspring, then we may find the percentage of likeness or homozygosis by the following equation:

$$\text{Homozygosis} = 50(1 + f_a)$$

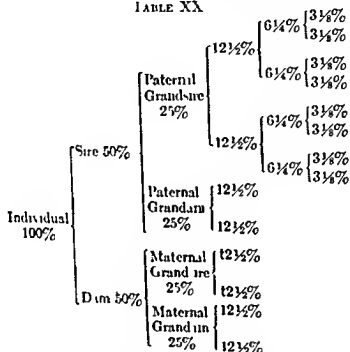
Thus, assuming 50 per cent of homozygosis from the parents from which X came, we have  $50(1 + .125)$  or 56.25 per cent; similarly, for Y we have  $50(1 + .4687)$  or 73.4 per cent homozygosis.

## ANIMAL RELATIONSHIPS

The term "family characteristics" is not an uncommon term. Breeders are often heard to remark about blood relationship of their animals and to measure it in terms of per cent. Relationship between

two animals is either direct or collateral. To be in direct relationship one must be the ancestor of the other. Collateral relationship of two animals exists when they have a common ancestor. Direct and collateral relationship may exist in the same pedigree if inbreeding has taken place in the line of descent. By 'blood' is meant inheritance generally. The term is not actually transmitted, for there is no transmission of blood from parent to offspring. Table XX shows an ex-

TABLE XX



ample of "direct" relationship arranged in the form of a pedigree in which the progeny may receive 50 per cent of his genes from parent, 25 per cent from grandparent, 12½ per cent from the next generation and so on back, the percentage being halved with each generation the ancestor is back in the pedigree. On account of the part chance plays in Mendelian inheritance, these percentages need not be exact for any ancestor back of the immediate parent. After we get back more than four or five generations, it seems practical to forget that any of these ancestors have contributed anything to the present generation. However, it is true that where some ancestors have contributed less, others must have contributed more.

Table XXI shows collateral relationship in the pedigree of the famous bull used in the New Jersey Breeding Experiment—N J E S

TABLE XXI \*

<b>Name</b> N.J.E.S. Sir Mutual Ormsby Jewel Alice #654594 <b>Born:</b> Dec. 1, 1931	Newmont Mutual Ormsby Lad 493889	Ormsby Sensation 45th 442551	Ormsby Sensation 274543
			Shady Maple King Pontiac Hockster 219580
	Newmont Ormsby Jewel Alice 1239022	Ormsby Sensation 45th 442551	Sir Mutual Scotia 170631
			Isa Scotia Beets 315248
			Ormsby Sensation 274343
			Shady Maple King Pontiac Hockster 219580
			Ormsby Sensation 45th 442551
			Clothilde Maid Ormsby Bess

\* From Rice, 1951.

Sir Mutual Ormsby Jewel Alice. This bull carries 62.5 per cent of the blood of Ormsby Sensation 45th, getting 25 per cent from the latter's appearance in the second generation of the pedigree and 12.5 per cent from his appearance in the third generation. If we examine the percentage of Ormsby Sensation blood in the two parents, we find Newmont Ormsby Jewel Alice carries 75 per cent and Newmont Mutual Ormsby Lad 50 per cent. Averaging the two, we get 62.5 per cent as the amount carried by N.J.E.S. Sir Mutual Ormsby Jewel Alice.

If we wish to find the relationship between the parents of an animal with a common ancestor, we measure the sum of all the possible paths by which duplication of gene effects in the common ancestor may have reached those parents. If we call one parent  $x$  and the other  $y$ , and recognize that there is an equal chance that contribution of each path is equal to  $(\frac{1}{2})^{n+n'}$ ,  $n$  is the number of generations from  $x$  back to the common ancestor and  $n'$  is the number of generations from  $y$  back to the common ancestor.

The sire and dam of N.J.E.S. Sir Mutual Ormsby Jewel Alice are collaterally related, that is, have a common ancestor, and the bull is closebred. Ormsby Sensation 45th may have transmitted some

characteristics through three different descendants to his double grandson.

To find the relationship of the sire and dam of "Alice," we must follow the lines to Ormsby Sensation 45th and count the generations involved according to the relationship formula:

$$R_{xy} = \Sigma[(1/2)^{n+m}]$$

From Sire to Ormsby Sensation 45th = 1

From Dam to Ormsby Sensation 45th = 1  $\therefore 1/2^{1+1} = 1/2^2 = 25\%$

From Sire to Ormsby Sensation 45th = 1

From Dam through Maternal Gran-

dams to Ormsby Sensation 45th = 2  $\therefore 1/2^{1+2} = 1/2^3 = 12.5\%$   
37.5%

This shows that the parents of "Alice" are 37.5 per cent related (small correction needed for inbreeding involved).

It will be seen that in direct relationship, blood percentage does express relationship; but when there is a common ancestor, it is a mistake to speak of blood percentage. The "Alice" bull is 62.5 per cent of Ormsby Sensation 45th. However, the sire which carries 50 per cent of Ormsby Sensation 45th and the dam 75 per cent bear a relationship of only 37.5 per cent to each other.

#### BREEDING METHODS COMPARED

The author would call attention to Table XXII, which is taken from a report of a recent study at the University of Maine (Hall and Dickey, 1957) in which outcrossing, inbreeding, and incrossing are compared.

TABLE XXII. RESULT OF MATING EACH OF 22 HOLSTEIN SIRES HAVING INBREEDING COEFFICIENTS OF MORE THAN 12.5 PER CENT WITH RELATED AND UNRELATED COWS\*

System	No of pairs	Dam's fat yield	Daughter's fat yield	Diff. (lb)
Outcrossing	373	468	492	+24
Inbreeding	114	498	466	-32
Incrossing	188	414	496	+82
Total or Av	625	457	459	+32

\* All records on 2X, 10 months' lactation, mature basis

## SUMMARY

This chapter has shown that there are several methods of mating animals to secure improvement. Grading up a herd or flock through sires used in artificial insemination units holds great promise for the beginner and for the commercial producer. It offers a more economical method than owning his own sire. Crossbreeding seems to offer desirable results in the production of meat and wool.

Close breeding does not create any new genes, but through re-combinations of good ones the breeder may concentrate desirable factors in his animals. Inbreeding produces good or bad results in a herd or flock, depending upon the hidden factors which exist in animals which have common ancestors.

Inbreeding can be successful only if we start with animals of the proper inheritance and practice rigid selection.

Only those breeders who have large herds or flocks and animals of above average quality should attempt inbreeding. Line breeding is safe and is recommended.

Finally, breed improvement requires skill, patience, and a fondness for animals.

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## Selection for Animal Improvement

---

JOHN W. BARTLETT

Better livestock and more economical production can be obtained chiefly by selecting the right kind of sire. We have often heard the saying that "the sire is half the herd." This is all too true when he turns out to be a poor one. Many livestock owners have been nearly ruined by sires whose daughters produced much less than their dams. *We should recognize that the sire has as much influence on the future females in the herd or flock as all the females to which he is bred.* It has been mentioned in previous chapters that artificial insemination permits the use of superior sires of known inheritance at a cost within the reach of any breeder.

The problem in sire selection is to get one whose inheritance is better than that of the top half of the herd or flock in which he is to be used. Even when the best judgment is used, sires will cross much better in some herds than in others. As a result of using proved sires for several generations, the United States Department of Agriculture Bureau of Dairying has very materially increased production in the Beltsville and substation herds. Experience indicates that best results are obtained when there is some similarity of blood lines in the sire and the females with which he is mated.

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## SELECTION BY RECORDS OR PEDIGREE

Selection, as has been shown previously, may be made on the basis of individuality, pedigree, or records of daughters. It is the author's belief that insofar as possible all three should be used. Every breeder should keep in mind the faults of his females when choosing a sire to mate with them. Certainly if there is a tendency toward sloping rumps, weak pasterns, poor shoulders, or unsound parts of any kind the sire should be especially strong in these respects. To base our choice of a sire on individuality alone is not genetically sound. What an animal looks like has been shown to be only a part of the story. His forebears may have been quite different.

After the individuality of the animal has been given the proper consideration its pedigree should be studied very carefully. To be of any real value a pedigree must be complete with information on the proper accomplishments of all the animals concerned. It should be noted that sire and dam may have the same genetic influence on their offspring, therefore a pedigree should be balanced. One should not put considerable emphasis on any one animal and neglect to realize that the animal mated to it may also have his or her influence.

Many dairymen in looking for a new sire are attracted to a flowery type of pedigree in which very little indication of his future performance is given. They are often led to pay prices all out of reason, knowing little of what his real inheritance is or what such a sire may be able to transmit.

Purchasing a future sire that is not already proved is always some what of a gamble yet we must select and prove young bulls. Table XXIII is an illustration of a pedigree in which the purchaser may have real confidence. Winterthur Zeus Fobes Crescent has two generations and more of ancestors that have demonstrated an inheritance for high production of milk and butterfat as well as very outstanding type. Recent reports on several daughters of Crescent indicate that he is transmitting very well when mated to females of high production and good type.

*What Is a Good Record?* Undoubtedly the greatest block to rapid improvement in breeding is created by the breeder himself. If he lacks willingness to get records of performance of his animals, he will lack information to guide him in his business.

TABLE XXIII PEDIGREE OF WINTERTHUR ZEUS FOBES CRESCENT

## PABST WINTERTHUR ZEUS

EX" Gold Medal Sire 931354

HIR Proof

28 HT daus 14 990 M 3 97% 595 F

27 HT daus 14,975 3 93 593

27 HT daus 15,629 3 94 615

-654 +.04 \*-19

Sire of

Winterthur Zeus Fobes Baska

4 y 3 m 2 X 365 20,436 3.8 774

Winterthur Zeus Fobes Cajalo

2 y 7 m 2 X 305 18 536 4 0 748

A National Record

Winterthur Zeus Fobes Cesequel

3 y 5 m 2 X 363 18 663 4 1 782

2d on 1952 Honor List

## WINTERTHUR ZEUS FOBES

CRESCENT 1024553 'VG'

Born October 10 1947

Breeder Winterthur Farms

Wilmington Delaware

## WINTERTHUR FOBES SELECT

ZAMALO VG' 2523493

3 y 4 m 2 X 358 14 643 4 0 586

4 y 6 m 2 X 305 16 267 4 0 647

5 y 8 m 2 X 350 27 745 4 0 703

6 y 10 m 2 X 365 15 405 3 9 602

4 y 10 m Classified

V V V + + + + +

A Daughter VG at 3 y 10 m

2 X 365 15 672 4 0 629

Zamalo a full sister

3 y 7 m 2 X 365 23 976 3 8 905

## WISCONSIN ADMIRAL BURKE LAD

VG' Gold Medal Sire 697789

Sire of All American Get 1947-48.

HIR Proof

105 daus 13,920 M 3 66% 509 F

63 daus 13 580 3 70 502

63 dams 13 000 3 62 471

+ 580 + 08 + 31

45 Excellent Offspring

Pabst Burke Lottie at 5 y 6 m 3 X 365

27029 M 3 8% 965 F

## WINTERTHUR GREAT KANN SOLOHR

1934437

2 y 3 m 3 X 365 16 217 3 8 609

3 y 4 m 3 X 365 19 035 3 9 747

4 y 7 m 3 X 365 24 857 4 0 997

6 y 2 m 3 X 365 24 398 3 8 929

She is a daughter of an

Excellent Sire

## POSCH ORMSB' FOBES 14th

'EX" Gold Medal Sire

HIR Proof

68 daus 15,380 M 3 87% 593 F

63 daus 15 400 3 90 601

63 dams 15 310 3 81 583

+ 90 + 09 + 13

Five times Leading Honor List

Sire of the Breed

## WINTERTHUR SELECT NEBSKA

ZAMALO

GP' 2205510

2 y 3 m 2 X 365 13 812 4 0 552

3 y 6 m 2 X 365 18,223 4 0 735

4 y 9 m 2 X 365 17,271 4 2 723

5 y 10 m 2 X 365 19 160 4 7 850

Five other records up to 11 years of age Her

sire a silver production sire

The dairyman may ask, "What is a good record?" He should not be satisfied with a few cows' records—his standard should be a yearly herd average. To be profitable the goal should be at least 400 lb. of butterfat a year on twice-a-day milking for two-year-olds. Such heifers should develop into cows that will produce 500 to 600 lb. at maturity and continue in production for five to seven years on the average. This is a high goal, but it can be achieved by continuous record keeping and the use of good bulls. Many dairymen have already surpassed this goal.

In selecting cockerels to mate with a flock of hens, trap-nest rec-

ords should always be used. The goal should be a flock average of 225 eggs per bird. Again this may seem high to the rank and file of poultrymen, yet there are numerous breeders who long ago passed this mark.

In hog breeding the sire selected should be from a sow that has from eight to twelve pigs and not from one that never has more than six.

*How to Evaluate Records.* We all like to look at big production records, yet we should evaluate those records. It is all too easy to confuse environmental influence with possible inheritance.

Those dairymen who milk only two times a day will rarely make an 800 lb fat record, but such a record is not infrequent among dairy cows that are milked three times daily. Cows milked three times each day, kept in box stalls, and fed the best feed that is avail-

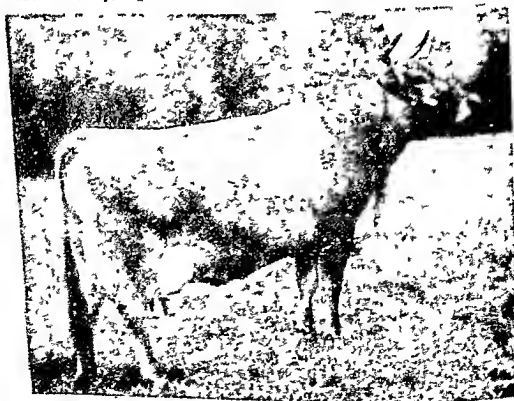


Figure 73 Lee's Hill Keeper's Raven holds the 1957 world butterfat record, over all breeds. Her dam and sire were second generation descendants of the great Brown Swiss, Jane of Vernon, and she herself is the result of line breeding. In seven lactations she has produced 180,498.6 lb of milk and 8,074.87 lb of butterfat. She is owned by Lee's Hill Farm, Morristown, New Jersey.

able at any price should not be confused with those which must depend mainly on pasture for their sustenance.

An 800-lb. fat record made on four milkings a day in 365 days is about equal to 675 lb. of fat on three milkings a day and 560 on two milkings a day. If the cow were to be milked 305 days or ten months and have a calf within the year, her record would be about equal to a 465-lb. fat record in the Dairy Herd Improvement Association program (sponsored by the United States Department of Agriculture in cooperation with the States) or the Herd Improvement Registry Test (supervised by Agricultural Colleges for the various breed clubs). It is evident that all factors would be equal under the above production comparisons.

High records are to be commended, but the average dairyman or any other breeder must not expect that high records under nearly perfect environment will be repeated under average farm conditions. It is advisable to consider how many records an animal has made. A cow that will produce 600 lb. of fat each year for three to four years under ordinary care is a great cow. A cow that will produce 800 lb. of fat in one year is a great cow; but unless she can do it more than once, the author believes the 600-lb.-fat cow is the best dam of a herd sire.

**Conversion Factors.** Cows will continue to increase in production of milk and butterfat from the time they freshen as two-year-olds up to the age of six to eight years, and thereafter their production will slowly decline. For the benefit of comparing records of bulls' daughters with their dams and of finding the mature equivalent of young or old cows, two sets of conversion factors have been worked out by the Division of Dairy Herd Improvement of the United States Bureau of Dairy Industry.

Table XXIV shows the age conversion factors reasonably applicable to all breeds of dairy cattle. Similar factors are available for the individual breeds and can be obtained from the Bureau of Dairy Industry. The mature equivalent record is found by multiplying the pounds of milk or fat produced by the factor opposite the age of the cow at the time her record was begun. Example: The record of a Holstein at two years of age was 10,000 lb. of milk. Her mature record equals  $10,000 \times 1.376$ , or 13,760 lb.

The United States Bureau of Dairy Industry considers that milking a cow four times a day increases her production 35 per cent and



TABLE XXIV

Age classification (years)	Single letter requirement	Conversion factor	Mature equivalent
5	400 0	×	1 00 = 400
4½	381 7	×	1 047 = 400
4	363 4	×	1 100 = 400
3½	345 2	×	1 159 = 400
3	327 0	×	1 223 = 400
2½	308 7	×	1 295 = 400
2	290 5	×	1 376 = 400

milking her three times a day increases her production 20 per cent over what it would have been on twice a day milking

*To convert**Multiply by*

A 365-day record to a 305 day record	0.85
A 305-day record to a 365 day record	1.17
A 4 times a day milking to a 3 times a day milking	0.83
A 4 times a day milking to a 2 times a day milking	0.74
A 3 times a day milking to a 4 times a day milking	1.13
A 3 times a day milking to a 2 times a day milking	0.83
A 2 times a day milking to a 3 times a day milking	1.20
A 2 times a day milking to a 4 times a day milking	1.35
To convert 2 milkings per day for 305 days to 3 milkings per day for 365 days add 40 per cent	
To convert 3 milkings per day for 365 days to 2 milkings per day for 305 days subtract 30 per cent.	

## PROVING THE SIRE

Today, though we talk about superior proved sires, there may be considerable difference of opinion as to what a superior sire is. It has been said that we must select a sire that will improve the top half of a dairy herd. A sire then may be a good bull in one herd but fail to increase production in a herd of higher average producing ability.

Artificial breeding has become big business during the past decade and will continue to increase as time goes on. During 1958 30 per cent of the dairy cows in the United States were artificially in

seminated. In some states over 40 per cent of the calves born are from artificially inseminated dams. As an artificial breeding bull may sire 40,000 or more progeny in a year and over 200,000 in a lifetime, it is very important that sires be carefully evaluated before selection.

Before considering the methods used in evaluating sires, the sire's own importance deserves mention. It has often been said that the bull is half the herd. In a herd in which ten to twenty or more calves are born each year the sire is thus ten to twenty times as important genetically as a cow in establishing the future of the herd. So it is with all sires in our animal industry. There is, however, no argument that both sire and dam are of equal importance from the standpoint of any one cow or bull. Each progeny always receives by chance assortment one member of each chromosome pair from each parent.

To evaluate dairy sires, then, we should have a proof of his influence on the cow population to which he is bred, or in other words a comparison of the production of his daughters with that of their dams, using the conversion factors shown previously in this chapter. In addition, many of our sire selection committees in artificial insemination organizations are giving attention to year and season of freshening. Records made in a dry year, for example, may not be a true indication of the production of a cow when on abundant feed. It has also been noted that cows that freshen in the fall produce more milk than cows that have their calves in spring.

Most bull proofs, then, are shown as dam-daughter comparisons, using the first five to nine dam-daughter pairs as a preliminary appraisal and ten or more pairs as better indication of a sire's transmitting ability. It can be illustrated as follows:

12 daughters av.	14,000 M.	— 4.0 —	560. F.
10 pairs daughters av.	14,200 M.	— 4.0 —	568. F.
10 pairs dams av.	12,800 M.	— 3.7 —	473. F.
∴ daughters increase +	1,400 M.	+ .03 +	95. F.

Of great importance is the level of production of dams of daughters to which a sire was mated. The breeder wants to know if the sire can maintain or increase production when bred to high-producing selected dams. There is much more to be desired in a sire who can maintain production at a 500-lb. fat level than a sire that raises production of his daughters to 400 lb. when mated, for example, to 350-lb. fat level dams.

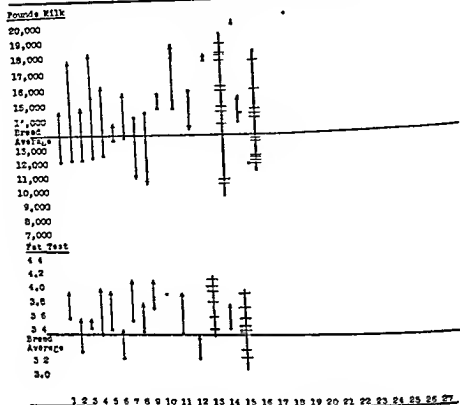


Figure 74 N J E S Mutual Ormsby Jewel Alice, 654594

12 tested daughters av 16,650 M. 4.2% 699 F

12 pairs dam av 15,470 M. 3.85% 596 F

As a further guide in studying a bull, the arrow chart may be used to show what a certain sire does under individual conditions. Figure 74 shows the arrow chart of N J E S Sir Mutual Ormsby Jewel Alice, 654594. The circle represents the dam's record, and the arrow the production or test of her daughter.

In only three cases did this sire fail to increase milk production, and in all cases he maintained or increased the butterfat percentage.

**The Artificial Breeding Proof Most Reliable.** It would appear that since bulls differ in their transmitting ability, an artificial insemination proof may be much more reliable than a natural proof, especially when the natural service proof is made in one herd. The recommended proof today is an artificial insemination proof showing the performance of 50 or more daughters of a sire used on several herds. Attention is called to Table XXV, which shows how natural service proofs of sires may change when the same sire is used in an artificial

TABLE XXV. NATURAL SERVICE PROOFS VS ARTIFICIAL INSEMINATION PROOFS ON HOLSTEIN BULLS

PAUL		OCAPOK		DEAN	
Number of animals	Pounds of butterfat	Number of animals	Pounds of butterfat	Number of animals	Pounds of butterfat
NATURAL SERVICE PROOFS					
20 daughters	517	32 daughters	428	30 daughters	497
14 pair daughters	505	30 pair daughters	416	30 pair daughters	496
14 pair dams	419	30 pair dams	372	30 pair dams	451
Difference	+86	Difference	+44	Difference	+45
ALL PROOFS CALCULATED BY CONVENTIONAL DAUGHTER-DAM METHOD (2X,305,ME)					
278 daughters	427	219 daughters	441	621 daughters	472
129 pair daughters	479	129 pair daughters	454	401 pair daughters	474
129 pair dams	417	129 pair dams	412	401 pair dams	417
Difference	+22	Difference	+42	Difference	+57
ALL DAUGHTER LEVEL ADJUSTED FOR YEAR, SEASON, AND HERD EFFECT AND THEN COMPARED TO BREED AVERAGE *					
278 daughters	434	219 daughters	453	1,071 daughters	466
Holstein Breed average	470	Holstein Breed average	430	Holstein Breed average	430
Difference *	+4	Difference	+23	Difference	+36

Source: Cornell Extension Bulletin 999 (1938)

\* Breed average = 2 X 305-day Mature Equivalent (M.E.) production average for all Holstein cows on DHI test in New York State for the past five years

insemination association. Adjustments are also shown depending on when dams and daughters made their records, and in comparison with the State Dairy Herd Improvement Holstein Breed average. The performance of three sires is shown.

**Other Methods of Sire Evaluation.** Some of our dairy breed organizations are now recognizing sires based on the average of their daughters' production when compared to the breed average, all records of course corrected for age, length of lactation, and number of milkings a day.

More recently a very promising method to evaluate young sires has been started in several states. This is called the Comparison of Stablemates. This method is not new, as it has been used in Europe for some time; however, it is proving of real value in getting an early appraisal of the transmitting worth of young sires in artificial breeding cooperatives. It is a method whereby the records of two-year-old daughters of artificial breeding sires used on several farms are compared with records of their stablemates. Records are on the basis of

2 × 305-day Mature Equivalent, taking into consideration that the season of year of freshening for those cows compared is the same. This type of study shows early in the use of a young sire whether he is maintaining, raising, or lowering production in several herds where the same environment exists between his daughters and their stable mates. The proof of one New Jersey sire is shown in Table XXVI.

TABLE XXVI SURVEY OF DAUGHTERS OF SIRE H-76

<i>H 76 Daughters</i> 17 Daughters			<i>Stablemates in 14 Herds</i> 315 Stablemates		
Milk ME		Fat ME	Milk ME		Fat ME
Av 12 120	3 6%	443	Av 12 740	3 7%	462
<i>Two-Year-Old Stablemates</i> <i>of Daughters of H-76</i> 79 Stablemates					
Milk ME		Fat ME			
Av 12 530	3 7%	461			

## SELECTING THE YOUNG DAIRY SIRE

Artificial breeding units as well as individual breeders must select and prove some young sires. The practice of breeding 500 to 1,000 females to a young dairy sire in a cooperative unit and then retiring him until he has milking daughters is certainly a safe procedure. In this way few daughters in any one herd will be born to him, and if he proves to be a failure, no one breeder will suffer from a group of poor producers.

In selecting the young sire the author suggests the adoption of the plan followed by most successful breeders in line breeding or sometimes inbreeding. In the New Jersey Artificial Breeding Program a plan of line breeding has been projected for some years to come. This system requires that some analyzed, related young bulls be used each year, as there will not be enough superior, related, proved sires to go around. Outcross bulls will also be in service.

Several factors have been mentioned previously in this chapter regarding standards to be adopted in selection of a proved sire. Individuality and pedigree are the principal guides in selecting the young unproved sire. It therefore behooves those responsible for the selection of the young sire to proceed with real caution.

*The female family should be studied.* The breeder should select a young sire that is a son of a superior proved sire; and he should be able to see representative paternal sisters. Since the dam may exert as much or more influence than the sire, he should also pay attention to the female ancestors or female family from which the young sire is to be chosen. Mark Keeney in his *Cow Philosophy* (1940) has most aptly said: "First and foremost the dam of our ideal bull will be truly a great cow. She will be great in her own producing ability. She will be great in the inheritance of her ancestors, and further, she will have demonstrated her ability to transmit greatness to her progeny." We believe that Mr. Keeney has demonstrated as well as any breeder that his great success as a Holstein breeder is expressed in this quotation. Table XXVII shows a dairy cow family that is consistent in production and test. The direct descendants of one cow are compared with their paternal sisters from four sires. The chances

TABLE XXVII

	Sire 1		Sire 2		Sire 3		Sire 4	
Dau's Av.	13,623	3.50	13,743	3.50	14,450	3.57	15,783	3.64
Dam's Av.	12,479	3.46	12,643	3.55	13,424	3.51	14,932	3.52

Family A Cow 96 7 Lac. 13560 3.56	112		139		163		214	
	6 Lac. 13573		5 Lac. 13890		4 Lac. 14978		2 Lac. 15962	
	3.58		3.60		3.57		3.75	
					178		269	
					3 Lac. 15642		1 Lac. 16840	
					3.64		3.81	
	117		147		168		206	
	5 Lac. 14723		5 Lac. 14963		4 Lac. 15630		2 Lac. 15641	
	3.60		3.65		3.72		3.73	
	122		150		183		271	
	4 Lac. 11504		4 Lac. 15423		3 Lac. 15756		1 Lac. 16300	
	3.64		3.68		3.74		3.78	

are good that this consistency may be passed on through a male born of this same family

The ability to reproduce, or in other words fertility, is a factor that is possibly inherited. In recent analyses of certain cow families extending through four to six generations, the tendency toward low conception was very evident in certain families. The number of direct descendants in a family of animals is a great criterion of the fertility of that family. The family of low fertility eliminates itself finally by natural selection. Certainly the breeder should avoid buying into such a line of inheritance.

The kind of feet and legs an animal has seems to be directly related to its ability to remain in production for a long time. Those whose feet are tender and whose legs are crooked and not well placed when standing may early have to go to the butcher. Therefore, type classification data of the ancestors of the males under consideration should be studied.

It should be evident from the recommendations in this chapter that one of the most important methods of selecting a sire is through records. The reader should understand that the breeder of livestock must select with greatest care his parent stock for succeeding generations, and discard without fear the inferior animals. Sires should be selected from pedigrees that tell a full story of production, type, and longevity of the ancestors. A sire's daughters are his best advertisement. Special cattle progeny stations were started in Denmark in 1945, and by 1954 there were 25 stations testing 92 progeny groups. Each progeny group consists of 20 daughters of a sire selected from one of several different herds. They must be twenty-seven to thirty-three months old and due to calve between September 20 and November 15. The groups are assembled at the stations on September 1 each year and the test covering the period from calving for each bull group is 304 days. Mason (1952) considers this sound genetically. Robertson and Rendel (1954) also report on artificial insemination calves in England and Wales. The Milk Marketing Board in England and Wales recently reported a comparison of artificial insemination bulls' daughters in testing stations with their contemporary sisters on farms. In two years of the four reported the sires ranked in the same order, whereas in the other two years there were discrepancies.

## OTHER LIVESTOCK INDEXES

*An Index for Meat Animals.* The breeders of meat animals are in greater need of a yardstick by which they may measure the inheritance of conformation than the breeders of dairy cattle. It is interesting to note that much progress is being made; still there is much variation in the transmitting ability of sires of all breeds of beef cattle, sheep, and swine. The show ring has done much to place emphasis on types that have particular market advantage. What the general livestock breeder wants in his sires is an inheritance to produce offspring that have high-quality meat and a tendency to develop economically. In other words, he wants an animal that grows rapidly into a symmetrical individual at a minimum feed cost.

Gregory (1933) proposed an index based on round measurement parallel to the ground from patella around the rear of the animal to the other patella. He called his formula  $RM/H$ , or round measurement divided by height at withers. The most typical low-set, beef-type animals measured approximately 106 cm. around and 123 cm. in height at withers, thus giving a  $RM/H$  reading of 862. The average dairy animal on the other hand gave a round measurement of 90 cm. and a withers height of 126 cm., or an index of 715. Gregory reported that the dividing line between beef and dairy was at an index of 785.

*Sheep Index.* The sheep breeder has a dual ideal in improving and maintaining his flock. His ewes must produce an annual crop of lambs of high meat quality, and the wool should pay the feed cost. Christgau (1939) at the University of Minnesota has reported that he uses the following plan as a measure of efficiency in selecting sires from a flock:

1. Record the ewe's weights, breeding dates, and fleece weights and grades.
2. Shear at the same time each year, and weigh ewes each year at shearing time and breeding time.
3. Record the lambing dates and lambs' weights.
4. Stamp the ewes' and lambs' numbers on the side of the body, using a system avoiding annual duplication. Stamp lambs at birth and ewes at shearing.



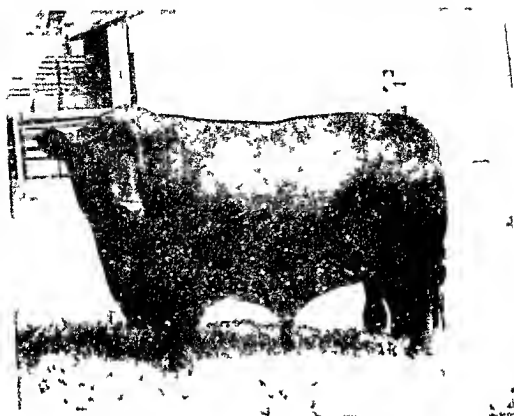


Figure 75 Worthy proved sire owned by the American Aberdeen Angus Breeders Association St Joseph Missouri

5 Weigh and weigh each ewe's lamb when fifteen weeks old

6 Weigh each ewe's lamb at twenty three months of age

7 Standardize all feeding breeding and management practices

All records should be kept on an individual ewe tabulation card to permit the breeder to identify the most efficient lambs to be used as breeders

**Swine Index** The problems in the selection of boars are not unlike those in the other breeds of livestock. Probably the greatest variation in swine is in growing ability and dressing quality. We shall not here emphasize nursing ability, fertility, size of litter, and longevity. Rice (1951) suggests an index for proving boars based upon sow performance and their litter comparisons. He recommends that a breeder save a certain number of sow pigs for breeding purposes. Record the birth weights of several, their weights at fifty six days and their daily rate of gain up to six months of age, then rate them as to market type on a scale ranging from 1 down to 10. We might

select six of these sows to mate to one boar. Table XXVIII shows an index on a good boar.

TABLE XXVIII. A BOAR INDEX DEvised BY PROFESSOR V. A. RICE AT MASSACHUSETTS STATE UNIVERSITY

	<i>Average birth weight</i>	<i>Average 56-day wt.</i>	<i>Average daily gain</i>	<i>Average grade score</i>
6 sows	2.4	32	1.1	4.3
40 pigs	2.6	38	1.3	3.8
Boar Index	2.8	44	1.5	3.3

#### A BREEDING PROGRAM IS NEEDED

The artificial insemination of farm animals has brought about a new era in animal improvement, just as the development of hybrid corn opened up new possibilities in the plant kingdom. To what heights we may go in average milk production in a dairy herd is not known, but we have many animals that now can boast of over 500 lb. of fat annually. It will be a long time before our national average approaches this figure. However, as livestock owners improve their animals they hope to keep on going up the ladder. Therefore if the organized artificial breeding is to remain on solid ground, plans must be made to attempt to find or breed superior sires capable of at least maintaining these new high levels of production.

Use of proved sires is of course the surest method of increasing or maintaining high levels of production. With the advent of artificial breeding organizations we began growing fewer bulls. Hence our field of selection has become narrower by the very fact that one sire can now take the place that 50 or 100 occupied in former days.

Sire committees of our various breeding associations must be impressed with the great responsibility that rests on their shoulders to find sires that can be depended upon to maintain the gains that have been made since 1940. They should have knowledge of animal genetics, and they should plan a long-time program and be ready to stand behind it. A few mistakes can make a lot of trouble. The program can be on an area, state, or multistate-wide basis.

In planning a long-time program certain facts must be kept in mind. (1) Not all members of any organization are going to be satis-

fied with each sire at the bull headquarters. Therefore, first the sire committee should strive to find the best proved sire that their funds will purchase. (2) Farmers today are becoming more and more type conscious. While type and production are not closely correlated, good type is worth money in sales of surplus animals. Type classification of purebreds today is a growing trend, and bulls that sire outstanding type are available. (3) When the average production of a group or herd reaches a high level, some line breeding seems advantageous in maintaining it.

These facts should be observed in a program that calls for improvement on a mass scale. Thus it should be the responsibility of a sire committee to enlist the cooperation of our best purebred live stock breeders for the sale, lease, or cooperative ownership of such proved sires as are available.

A plan for herd analysis and for accumulating and studying records of young bulls' daughters should be a part of the long time program.

A plan for stabling young sires while not in use should be worked out. It seems best that once a young sire has been used for artificial insemination he should never be used for natural service. This is not necessarily a rule, but it is a good plan to follow.

#### SUGGESTIONS FOR SIRE SELECTION

1 Sires should be selected on a production and type (herd analysis) basis.

2 Proved sires should have offspring with records commensurate with the herds in which the sires are to be used. Study an arrow chart of each sire's daughters.

3 Pedigree, production, and official type classification records of ancestors should be consulted in sire selection.

4 All production records, when being used in sire selection, should be standardized to common denominator, namely (a) Mature Equivalent, (b) 305 days, (c) two milkings daily.

5 Blood lines are not important, but some line breeding is recommended when purchasing or putting new sires into a breeding program.

6 Fertility of the proved sire as shown by previous breeding records should be noted. Only young sires from fertile families should be used.

7. All young sires should be mated with enough tested females to assure 50 dam-daughter comparisons.

8. Special quarters for young sires should be provided while they are being proved. Natural service in farmers' herds is not recommended.

9. Health is essential. Freedom from tuberculosis, brucellosis, trichomoniasis, and vibriosis should be required.

10. A long-time program in the hands of competent sire committees is essential.

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## Artificial Breeding Organizations

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ENOS J. PERRY

Mass artificial insemination of farm animals, particularly cattle, is now being practiced in a good many of the countries of the world through artificial breeding organizations. Several of the groups failed in the beginning, but most of them have progressed, many combining to form the larger centralized type of organization. These are playing an increasingly important role in the improvement of cattle. With the most careful type of management they will continue to grow in proportion to the amount of constructive service rendered.

In 1961, 7,047,148 dairy cows were bred in the artificial breeding organizations operating in the fifty states and Puerto Rico. This number, the largest in any year since the beginning of the artificial breeding in the United States, was 36.7 per cent of the cows and heifers two years of age or more kept for milk during 1961. These cows were located in an estimated 863,781 herds.

Table XXIX shows that the average number of cows bred annually per sire has increased from 228 in 1939 to 3,010. Note the increase in proved sires and in the production level of their tested daughters since 1943.

In eleven states 50 to 60 per cent of the dairy cow population was bred artificially in 1961. These states were New Hampshire, Connecticut, Maine, Massachusetts, Washington, Utah, Idaho, New Jersey, Delaware, Pennsylvania, and Wisconsin. The largest pro-

TABLE XXIX. STATUS OF ARTIFICIAL BREEDING PROGRAM IN THE UNITED STATES, 1939 TO 1962

Year	No. studs	Sires in service			No. herds *	Cows bred		Dairy sires in service					
		Total no.	Proved (%)	As. per stud		Total no †	As. per sire	Total no.	(prod. data—lb.)				
									Dams			Daughters	
									Milk	Fat		Milk	Fat
1939	7	33	—	4.7	646	7,359	228	—	—	—	—	—	
1940	25	138	—	5.5	2,971	33,977	246	—	—	—	—	—	
1941	33	237	—	6.8	5,997	70,751	299	—	—	—	—	—	
1942	45	412	—	9.0	12,118	112,788	274	—	—	—	—	—	
1943	59	574	23.5	9.7	23,448	182,524	318	135	9,559	387	10,155	419	
1944	58	657	19.6	11.7	28,627	218,070	332	129	9,953	391	10,490	421	
1945	67	720	20.2	10.9	43,998	360,732	495	147	9,629	387	10,488	428	
1946	78	900	23.2	11.6	73,293	537,375	697	209	10,270	401	10,741	423	
1947	84	1,453	23.1	17.3	140,571	1,184,163	615	335	10,099	405	10,830	430	
1948	91	1,745	24.4	19.2	224,493	1,713,581	932	426	10,161	407	10,575	433	
1949	90	1,940	24.6	21.6	316,177	2,091,175	1,078	514	10,157	412	10,499	435	
1950	97	2,104	26.6	21.7	409,300	2,619,555	1,245	559	10,235	416	10,734	444	
1951	94	2,187	29.0	23.3	548,300	3,509,573	1,605	634	10,267	419	11,009	459	
1952	94	2,324	29.6	24.7	671,100	4,295,243	1,845	698	10,510	422	11,210	471	
1953	95	2,596	36.7	27.1	753,000	4,845,222	1,863	953	10,375	429	11,175	473	
1954	93	2,661	36.1	28.5	805,000	5,155,240	1,937	960	10,483	432	11,239	475	
1955	79	2,430	36.0	31.0	845,900	5,413,674	2,210	833	10,575	436	11,265	476	
1956	79	2,553	36.6	32.2	900,400	6,762,656	2,257	932	10,600	438	11,301	477	
1957	75	2,651	40.5	35.3	946,000	6,035,982	2,284	1,074	10,625	440	11,394	477	
1958	71	2,676	37.8	37.7	975,372	6,645,563	2,483	1,012	10,772	446	11,345	478	
1959	64	2,460	39.1	35.4	930,059	6,932,294	2,816	963	10,916	451	11,493	482	
1960	62	2,544	18.8	41.0	910,000	7,144,679	2,808	477	10,790	437	10,792	443	
1961	65	2,486	19.6	44.4	853,781	7,482,740	3,010	433	11,181	445	11,172	452	
1962	66	2,456	—	43.9	—	—	—	—	—	—	—	—	

Source: National Cooperative Dairy Herd Improvement Program. *Dairy Herd Improvement Letter*, XXXVIII (March, 1962). (Agricultural Research Service, U. S. Dept. of Agriculture.)

\* Estimated for the years 1950-1961. † Prior to 1946 cows were reported only on the basis of enrollment.

gram was Wisconsin's, with 1,291,835 cows bred in 1961. Other states with more than 300,000 inseminated that year include Minnesota, New York, Pennsylvania, Ohio, California, and Michigan.

**Beef Bulls in the Artificial Insemination Program.** Since 1950, a surprising development has occurred in the request for the service of beef bulls, both by the dairy cattle owners and the all-beef raisers. A total of 420 beef sires was used by 45 of the 56 artificial breeding studs of the United States in 1961. The number of beef cows bred was 435,592. During the same year, 38 per cent of the calls for artificial insemination service in England and Wales was for beef and dual-purpose bulls, to inseminate a total of 752,860 cows. (Edwards,

TABLE XXX. LEADING ARTIFICIAL BREEDING ORGANIZATIONS IN THE UNITED STATES, 1961

<i>Stud</i>	<i>No. cows bred</i>	<i>No. bulls used</i>
American Breeders Service, Madison, Wisc.	1,431,462	126
Curtiss Candy Farms Improved Stud Service, Inc., Cary, Ill.	817,216	150
New York ABC, Inc., Ithaca, N. Y.	553,067	123
Badger Breeders Cooperative, Shawano, Wisc.	373,007	68
Minnesota Valley Breeders Assn., New Prague, Minn.	321,496	69
Tri-State Breeders Cooperative, Westby, Wisc.	421,464	99
Michigan ABC, Inc., East Lansing, Mich.	259,243	94
Central Ohio Breeding Assn., Columbus, Ohio	240,012	63
NOBA, Tiffin, Ohio	218,820	78
Consolidated Breeders Cooper- ative, Inc., Anoka, Minn.	242,610	53

Source: National Cooperative Dairy Herd Improvement Program, *Dairy Herd Improvement Letter*, XXXVIII (March, 1962). (Agricultural Research Service, U. S. Dept. of Agriculture.)

1960-61.) This greatly increased demand has come about chiefly because: (1) A few top sires have been made available to owners of purebred or high grade beef herds in a state or region. (2) Those owners have been enabled to do a better job of controlling breeding troubles. (3) Dairymen have been afforded the opportunity to have their first calf heifers bred for easier calving, and then raise the resulting crossbreds for beef or veal.

#### ARTIFICIAL BREEDING IS BIG BUSINESS

From the foregoing it is obvious that artificial breeding has become big business. Its development in many countries has been more rapid than was anticipated during the initial stages from 1937 to 1943. The research staffs of government experiment stations and



Figure 76 America's first artificial breeding association at Clinton New Jersey began operations in cattle insemination in May 1938. A two-way radio system directs technicians to farms where cows have reached the optimum stage of heat for breeding.



of certain commercial companies have made discoveries without which the movement would have advanced slowly. Many of the groups emerged as large enterprises requiring managers of exceptional ability and officers and boards of directors elected from among the top leaders in the membership. A little later, private industry was attracted by the business success of the cooperatives. In January, 1959, there were 64 active cattle inseminating studs in the United States, and several of these were commercial in nature.

Today commendable artificial breeding work is being done both by the cooperatives and the private, commercial corporations. The sole criterion should be the quality of the service rendered. Some livestock owners believe that the cooperative is likely to function more economically and that its continued existence over a long period is not so likely to depend upon the aggressive leadership of one or two individuals. A true cooperative develops leadership among its members. Responsibility for improving its efficiency is continually being delegated to different committees.

On the other hand, if any private breeding unit is in a position to offer continuous service of a high order, covering all phases of an inseminating program, it will likely gain a fair share of the patronage of the livestock owners, in keeping with the spirit of free enterprise.

Contemporary with the multiplication of airlines and ever faster plane service has come the competitive bidding of some of the large breeding organizations for the business of livestock owners living far off, sometimes in foreign countries. But the biggest factor has been the improvement in the techniques of freezing and shipping semen, especially bovine semen. With many more ampoules of semen potentially available from great sires than are needed by these organizations, it is natural for them to seek distant markets. A United States breed journal recently carried large advertisements, mostly full-page, by 14 of these organizations, some cooperatives and others private.

#### EXPANSION OF ARTIFICIAL INSEMINATION PROGRAMS

The artificial insemination organizations that have grown most rapidly in many countries in the past twenty years are those that have performed a type of service over and above that initially expected by the members. The managers and technicians have ap-

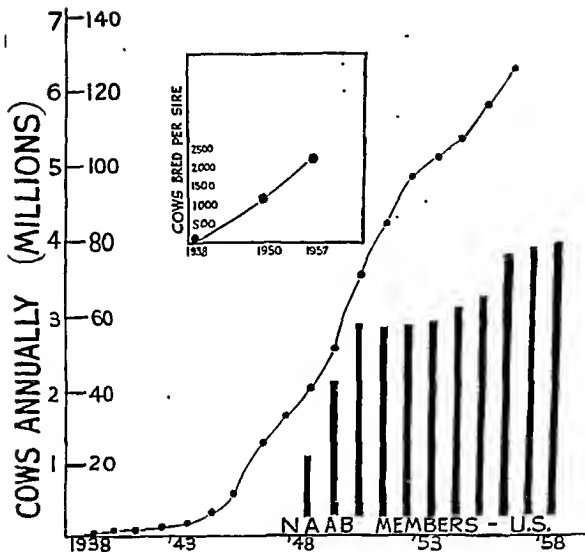


Figure 77. Growth of artificial breeding of cattle in the United States. (Courtesy A. I. Digest.)

preciated the herd owner's year-to-year problem of having born on his farm, young stock that are satisfying both in numbers and quality.

A well-conducted field service is very important in expanding the growth of a breeding organization. This source can be very broad. Its scope and effectiveness depend on the imagination and competence of the board of directors and manager and the support given by the entire staff. In its province fall such activities as publicity, cooperation in health control, production testing and culling, progeny appraisal, the holding of demonstrations and open house, the setting up of special exhibits, and participation in local, district and state cattle shows.

The salary of a technician is sometimes a fixed monthly or annual wage. It can also be based on a minimum wage with a bonus incentive for increasing the volume of business. This encourages

the technician to do work of a high order and to solicit new customers as opportunity permits

**Reducing Semen Costs** The chief ways to reduce the cost of semen per cow inseminated are (1) Maintain highest quality standards in producing, processing, and shipping (2) Increase the number of cows serviced—the volume of business (3) Cut all unnecessary costs

When breeding efficiency is high, the required amount of semen is kept at a minimum. An increase in cows inseminated is a key factor in keeping an organization in the black. Any attempt using various "gimmicks" to bolster an unscientific type of program is doomed to fail in achieving even a temporary pickup in business.

#### THE NATIONAL ASSOCIATION OF ARTIFICIAL BREEDERS

The National Association of Artificial Breeders of the United States, commonly known as N A A B, was founded in 1947. Its objective is to aid in giving purpose and direction to the organized artificial breeding of livestock and to cooperate with individual breeders and with various agencies, such as breed associations, state colleges of agriculture, veterinary colleges, the state and federal departments of agriculture, and the foreign agricultural service, in expanding and improving the project of artificial insemination. From a small nucleus of a few breeding groups it has grown rapidly until in 1961 the membership had reached 115 organizations, which included 102 members and associate members in the United States and 13 associates in other countries. The history of N A A B has been characterized by actively working committees, well programmed annual meetings, the publication of a highly informative monthly journal, *The A I Digest*, and the fruitful achievements of an executive secretary. Much assistance has been rendered in the standardizing of techniques, that is, in the handling, preserving, and use of semen, in record keeping, and in the management of bulls.

The organization's budget for 1961 was \$117,566, of which approximately \$30,000 was for research relating to such ever-new problems as sire selection, semen evaluation, and its processing and utilization. Today, the managers and technicians of most of the artificial breeding groups have jobs with career status. N A A B has assisted immeasurably in demonstrating the eminently worthwhile vocational as well as scientific aspects of the artificial insemina-



Figure 78 Headquarters of England's Milk Marketing Board, Thames Ditton, Surrey  
Its 22 artificial insemination centers are breeding more than half of the national herd

tion program To date the organization has concerned itself chiefly with cattle. Its motto is "Better Cattle For Better Living" Its code of ethics, adopted September 24, 1952, follows.

#### CODE OF ETHICS—NATIONAL ASSOCIATION OF ARTIFICIAL BREEDERS

1. All members shall conduct their affairs to carry out the aims and objectives of the National Association of Artificial Breeders
2. No sire will be purchased under any agreement providing extra payment if his daughters in the owner's herd achieve a stipulated production level.
3. No sire will be put into service for payment of an additional service fee as a condition of registration of his offspring.
4. The source of daughter-dam comparisons, or daughter-averages, will be indicated whenever published.
5. In published daughter-dam comparisons, or daughter-averages, no daughters with completed records of 305 days or less will be omitted
6. Publication of non-return information will be on a 60 to 90-day basis and will be labeled as such.

- 7 No good purpose is accomplished by extravagant claims of the merits of sires, or of the efficiency of organizations. All members will use truthful statements concerning their own association and other associations.
- 8 No organization shall employ a person who has been dishonest or willfully negligent with the rules of the Purebred Dairy Cattle Association.
- 9 It shall be unethical for any member of this organization to attempt to interfere with techniques and practices developed by another organization, whether such interference be by unfounded criticism, interference with or by the proselyting of techniques employed by other organizations, or in any other manner unfairly disrupting another organization.
- 10 All statements made by an organization concerning its, or another organization's business, shall be susceptible of proof by adequate evidence, and all such evidence as well as evidence bearing on any violation of this Code shall be submitted to N A A B or authorized committee, thereof, upon request by N A A B.
- 11 If any of the provisions of this Code shall violate any State or Federal law, neither N A A B nor any of its members shall be bound thereby or shall be deemed to have agreed thereto.

#### TWO TYPES OF ARTIFICIAL BREEDING ORGANIZATIONS

The two common types are the local breeding organization and the central or federated organization. They are largely interdependent.

The *local* is usually a cooperative association for a county or for several adjoining counties, although it may be privately owned and operated. Breed associations, milk producers' cooperatives, creameries, or banks may sponsor the setup. However, very few of these locals are able to continue on an independent basis due to high overhead costs per cow serviced. They usually affiliate with a central or federated group, enjoy an improved financial condition, and obtain the service of superior bulls made possible by a large volume of business.

The *central* or *federated* organization operates on a state-wide or large district basis. It may be a cooperative or private enterprise such as that now functioning successfully in many states. The semen used by all of the "locals" is produced at the central headquarters and sent by mail, bus, plane, or special carrier two to six times per week, in the case of liquid semen.

Whether a local obtains semen from a state-wide cooperative or from a private commercial company, there must be cooperative effort and responsible officials to transact business. Certain steps pertaining to organization and management must be taken. The most important of these with reference to the *basic local unit* are:

1. Election of outstanding officers and directors by the members.
2. Payment of a membership fee and (or) a fixed entrance fee per cow to assure adequate funds for buying the required equipment and providing a working fund.
3. A breeding fee set high enough (for three breedings if necessary) to cover the costs of service without resorting to yearly dues.
4. Operation of the business on a cash basis.
5. Complete record keeping on breedings and finances.
6. Employment only of technicians who are thoroughly trained, and who are deeply interested in the problem of herd improvement.
7. No extension of special breeding privileges to anyone, and adherence to the decision of the central to use the bulls in scheduled rotation insofar as that system is workable.
8. After affiliation with a central or federated cooperative, assumption of the expected share of responsibility for its successful operation



Figure 79 The Centra de Las Angeles in Chile, which serves an area 200 miles long  
(Courtesy U S Operations Mission, Chile)

Whether planning a local or a state-wide, regional or national, setup, it is always advisable to inspect in detail the operations of a similar organization, talk with the manager, and study the by-laws and regulations, and especially the financial and business arrangements.

In order to keep operating expenses of the association at a minimum, the headquarters should be located as near the center of the area to be served as is practical. The more concentrated the cows, the less travel will be required of the technician. Also, the telephone cost to the members in reporting cows to be bred will be at a minimum. The laboratory equipment required need not be elaborate but must include an electric refrigerator, microscope, and facilities for providing hot water for cleaning the equipment.

**The Central.** All members of the locals are also members of the central association which owns or leases the bulls, bull barn, and laboratory. All the bulls are kept at the central bull barn. The central association should be incorporated, and the locals with it, as affiliates or subsidiaries. The semen used in all associations is produced at the central bull barn and mailed or sent by bus or other means to the local associations. The local associations usually employ their technicians and manage the affairs of the local group. Directors for the central association are elected by the locals, sometimes in proportion to the number of cows serviced per year.

This type of organization is particularly suited for the expansion of the artificial breeding program. New locals can affiliate themselves with the central association as they are set up.

**Financing.** Proper financing of both the central and the local organization is of utmost importance. The following provisions from the by laws of the New York Artificial Breeders Cooperative, Inc., are fairly representative of those organizations that are enjoying a sound financial position.

**ARTICLE 8—CAPITAL Section 1—Membership Fee.** In order to provide capital each member shall pay a membership of \$— in the Corporation and \$1— for membership in each local association from which he receives service.

**Section 2—Assessment.** In order to provide additional capital each member shall pay a nonrecurring assessment of \$— per first service in each herd for which he receives service from the Corporation during any fiscal year of his local association.

**Section 3—Return of Capital:** The membership fees and assessments shall constitute capital of the Corporation, and the Corporation shall maintain records to show the capital contributions of each member. When the Corporation has funds in excess of its needs, capital may be returned to the persons who invested it, or their heirs, or assigns, by action of the board of directors in any one or more of the following ways:

a) The membership fees and assessments of persons no longer members may be returned in the order in which they were invested.

b) All of the assessments invested in any year may be returned, provided that the assessments invested in all preceding years also have been or are concurrently returned.

c) All of the membership fees invested in any year may be returned, provided that the membership fees invested in all preceding years have been or are concurrently returned.

**ARTICLE 9—OPERATING FEES. Section 1—First Service Fee:** Each member shall pay a service fee of at least \$— before each cow is bred, of which part shall be payable to the Corporation for operating expenses and part to the local association for its expenses.

**Section 2—Additional Service Fee:** Each member shall pay an additional service fee of \$— for each service after the third in any lactation period, of which part shall be payable to the Corporation for operating expenses and part to the local association for its expenses.

**Section 3—Rebreeding After Six Months:** Except for heifers which have never freshened, any rebreeding six months or more after the last previous service shall be regarded as a new first service for that cow and a first service fee shall be collected.

**Section 4—Division of Service Fees:** The local associations shall be considered to be the patrons of the Corporation.

The division of the service fees between the Corporation and the local associations shall be determined by the board of directors. (By action of the board of directors, the Corporation may return to the district associations part of its share of service fees already collected.) The foregoing provisions shall be applied uniformly to all local associations.

**Section 5—Net Savings.** Any net savings remaining from service fees and income at the close of each year, after provision for all expenses, shall be allocated to the patrons in proportion to their contributions thereto.

**Section 6—Audit:** Immediately after the close of each fiscal year, the Corporation's operations shall be audited by a public accountant. A report of the operations of the Corporation for the fiscal year together with a statement of its financial condition shall be submitted to the annual meeting of the Corporation. Within one month after the annual meeting,



the Corporation shall file with the Department of Agriculture and Markets, a copy of its annual audit, together with a statement of the names of its officers and directors, the number of members admitted and withdrawn during the year, and the total number of members

Finances are budgeted in two divisions capital investments and operating costs

Capital investments include such items as cost of bulls, equipment, a farm on which to keep bulls, paddocks, laboratory, and so on. These long-time investments, referred to as capital costs, are paid for by membership fees, advances or loans from members, such as assessments for each cow, usually one dollar per cow, extra charges for first services, and so on, endowments from persons, foundations, organizations, support from state or county farm groups, or loans from local banks, banks for cooperatives, production credit associations. Most newly formed associations err in incurring debts beyond the means of the association to repay, and in assuming unsound financial obligations.

Operating costs include salaries and reserve funds for bull replacements, depreciation of the physical plant, bull feed, semen shipping costs, and so on. These costs are financed from the breeding fees. The breeding fee should be sufficient not only to cover operating costs but to build up a reasonable reserve fund, and in some organizations to retire the capital debts when so financed.

The work of the central is greater than many of the members of the Locals sometimes realize. The Michigan Artificial Breeders Co-operative lists the following as among the costs and items it pays for: labor, feed, bedding, barn supplies, office supplies, matching of the Social Security sum paid by the technicians and central employees, bonding of technicians and local secretaries, insurance, dues to National Association of Artificial Breeders, blood typing of bulls, calendars, bull manual, movie film of operations and results, fieldman, exhibits to fairs, newspaper mats, newsletter, receipt books, mimeographed material for meetings and the research program.

**Approved Associations** In the United States, the Purebred Dairy Cattle Association, the National Association of Artificial Breeders, and the American Dairy Science Association have cooperated in establishing requirements governing the insemination of purebreds in all of the artificial breeding organizations. An approved "business" is one that has been formed in accordance with sound methods of

organization, has provided for adequate record keeping, financial records, and others. Approved artificial breeding organizations are required to file authorizations of signature and a signature card for their manager, and for each technician in their employ, with each breed registry association with which their members will file applications for registry.

The member breed clubs of the Purebred Dairy Cattle Association are:

Ayrshire Breeders' Association  
Brandon, Vermont

Brown Swiss Cattle Breeders' Association  
Beloit, Wisconsin

The American Guernsey Cattle Club  
Peterboro, New Hampshire

The American Jersey Cattle Club  
Columbus 5, Ohio

The Holstein-Friesian Association of America  
Brattleboro, Vermont

The officers, manager, and technicians of every cattle breeding association artificially inseminating purebred, registered dairy stock in the United States must be wholly familiar with the contents of the booklet, "Requirements Governing Artificial Insemination of Purebred Dairy Cattle." This set of regulations is obtainable from any of the above member clubs of P.D.C.A.

#### AN INFORMED MEMBERSHIP

A well-informed membership has been one of the important factors in the breeding organizations noted for their rapid growth. Copies of pedigrees of new bulls in service should be sent out promptly, together with any special news about them. Furthermore, it is important that timely items and educational facts, based on recent happenings, be issued periodically. The large-scale breeding operations afford data that are of intense interest and carry lessons that are understandable.

**News Publications Popular** More and more of the larger organizations are doing an excellent job of keeping their members well informed through the medium of monthly or quarterly publications. These are usually four or eight pages. They contain notices of coming events, reports of interesting developments, detailed facts about new bulls, and complete data on those in service that were recently proved. The names of some of these publications are *Progressive Breeder* (Maryland), *The KABA News* (Kentucky), *Test Tube Tales* (Maine), *Bull Tales from K A B S U* (Kansas), *Test Tube News* (Northern Ohio), *New York Artificial Breeders' Co Operator*, and *The Breeders' Broadcaster* (Southeastern Pennsylvania).

**Figuring Breeding Efficiency of Bulls** Under field conditions, the estimates of breeding efficiency are nearly always based upon non returns—sometimes wrongly called conceptions. These are figured for various intervals, the most common of which are 60 to 90 days, and 90 to 120 days.

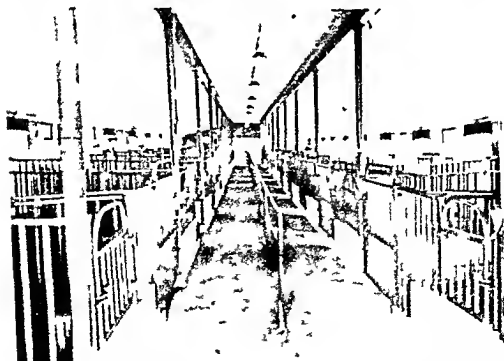


Figure 80 One of the modern bull barns at Southeastern Pennsylvania Artificial Breeding Cooperative, Lancaster. Each of the 20 box stalls is 12 by 14 feet. Stall doors are operated by ropes and pulleys for safe handling of bulls. Outdoor exercise pens measure 12 by 35 feet with a 10-foot alley between them.

Casida, Barrett, and Lloyd (1946) made a careful comparison of conception as determined by pregnancy examination with the conception as estimated from the breeding record reports of the inseminators. They found a discrepancy of 15.3 per cent at 30 to 60 days, 6.1 per cent at 60 to 90 days, and 3.0 per cent at 90 to 100 days. The majority of the diagnoses were made by palpation of the amniotic vesicle between 34 and 50 days after breeding. Before the discovery of antibiotics, a 60 per cent non-return rate was considered excellent in any breeding organization. An average yearly rate of 70 per cent is now no longer extraordinary.

**Housing and Exercise.** In selecting a site and planning the housing facilities it should be remembered that it may be necessary to provide for many mature bulls and have additional room for future expansion. Electric exercisers are used by some associations and are considered beneficial, especially for those bulls that are naturally inactive. Every barn arrangement should include a breeding rack under cover.

**Safety Devices.** Every bull should be considered dangerous. Most associations have installed some type of arrangement for the special protection of the caretaker and the technician at time of collection of semen. Good devices include a fenced alleyway in which the bull goes to the collection point under the control of the caretaker on the outside. Every inside pen should have a good strong stanchion in which the bull can be fastened for easy catching, and for grooming and treatment.

#### PROBLEMS FACING ARTIFICIAL BREEDING ASSOCIATION

The most important problems that face artificial breeding organizations are these:

1. Obtaining more good proved bulls (that are fertile), either by association proving or by purchase.
2. Prolonging effective usefulness of bulls and preventing fluctuation in breeding efficiency.
3. Correcting the impression in some areas that artificial breeding is a method to use when natural service fails to produce conception.
4. Investigating a too low non-return rate in certain herds where conditions appear to be excellent.

5. Developing a proper appreciation of the part that good inheritance can play in herd improvement.

#### PUNCH CARD ACCOUNTING IN BREEDING ASSOCIATIONS

The necessity of using a modern means of record keeping cannot be overemphasized. A gold mine of information, most of it essential, the rest highly useful, is provided by a punch card system of the type that the larger breeding organizations have installed in various countries. The method of operation and the great advantages accruing are outlined below in a report by Robert A. Bruemmer (1959), office manager, Southern Illinois Breeding Association:

"In breeding associations that breed over 50,000 cows per year it seems practical to adopt a punch card system of accounting and statistical work than to resort to any other system of record keeping. It is necessary to have at least 50,000 cows per year because the cost of this system, with the basic machines necessary, is about \$750 per month or \$9,000 per year. This same basic system which includes an 026 IBM key punch, 024 IBM verifier, 082 IBM sorter, 077 IBM collator, 403 IBM accounting machine, and 514 IBM reproducing punch will easily handle 125,000 first services per year, and with the addition of another 026 key punch will probably handle well over 200,000 first services per year. Three people are necessary to operate the basic system, and four would be necessary to operate the slightly enlarged system with the additional 026 key punch.

"The Southern Illinois Breeding Association is a good example of one that is using the above-described system. Before IBM machines were adopted, all billing, sire analysis, technician efficiency, monthly operating reports, member equity records, and any other records were kept by hand. It was necessary to have nine girls in the office, and records were usually behind. Since the switch to IBM, two girls and the office manager do all of the work formerly done by the nine girls, and reports are about a week or ten days ahead of the old system.

"IBM can do the work much faster and cheaper than the hand method because one punched card can be used for all records.

"When setting up the IBM system the first step is to code all information to be punched into the cards. Members must be assigned account numbers, and bulls and technicians must be assigned code



numbers. These numbers eliminate a lot of punching and speed up the operation considerably. For example, it is much faster and simpler to punch bull number 1-36 than to punch the name U N H Quartermaster, and to the personnel the number 1 means that it is a Holstein, and the number 36 is Quartermaster, thus 1-36 = U N H Quartermaster.

"At the Southern Illinois Breeding Association all technicians send in a daily report. This daily report gives the office all information, in code, in the same order that it is to be punched into the IBM card. Two changes have been necessary in the original card design. Immediately after these cards are punched, they are sent to the verifier where a different girl punches the same information. If the cards are correct, they will go through the machine and a notch will be cut in the end of the card and it will be ready for further use. After all of the cards have been punched for all sales areas for one day, a daily audit and daily accounts receivable register can be run. These reports must agree with tape totals taken from the original daily reports sent in by the technicians.

"The daily audit now run on the 403 accounting machine, shows the sales area no., member account no., breeding receipt no., registration or ear tag no., amount of charge, breed of cow, if cow is registered or grade, bull used, present technician no. breeding the cow, service no., date of previous service, previous bull used, code no. of previous technician, repeat code, and in the first card of each day the total no. of miles driven for that particular sales area. The totals on this daily audit must agree with the tape totals on the daily reports.

"After the daily audit is run, the first service cards are separated from the repeats on the 082 sorter. The first service cards are then sorted by account no. within sales area. The repeat cards are reproduced on the 514 reproducer transposing service date, and previous service date, bull used, and previous bull used, and technician, and previous technician. The original repeat cards are filed in the member's herd record, and the reproduced cards are filed for further use. The first service cards are reproduced just as they are. The reproduced cards are filed with the reproduced repeats, and the original cards are sent to the 403 accounting machine where an accounts receivable register is run.

"The accounts receivable register shows the member's name, ear

tag or registration no. of the cow, sales area, member's account no., date of service, and amount of charge. The total charges must again agree with the tape totals from the daily report. These cards are then taken to the 077 collator, where they are merged into an open item file consisting of all charges not paid by members.

"The payments that came in with the daily reports are sorted into account no. within sales, and charges in the amount of these payments are pulled by hand from the open item file. These cards are then sent to the 514 reproducing punch, where the date paid is punched into them. After this they are sent to the 403 accounting machine where a cash receipts register is run.

"The cash receipts register shows the member's name, ear tag or registration no. of the cow, sales area, member's account no., date of payment, and amount of payment. The total payments must also agree with the tape totals from the daily reports. At the end of the month all cards remaining in the open item file are merged on the 077 collator with a master name and address file, and statements are then prepared on the 403 accounting machine. The daily audit, accounts receivable, cash receipts, and billing steps are necessary only in offices that have centralized billing and can be eliminated in any organization that has billing on a local basis. For the most efficient operation it seems that the basic machines described are necessary even if billing is eliminated.

"At the end of each month it is a simple matter to run an operating report showing first services, 2 $\times$  services, 3 $\times$  services, and 4 $\times$  and over services by breed for each sales area. This is done by sorting on the 082 sorter all reproduced cards, first by breed of bull used, then by sales area.

"After the operating report is completed, the cards are again sorted by date and then by bull number. After this, the cards are taken to the tabulator, and breeding efficiency is run by bull by day, and summary cards are punched and run through the tabulator, and the sire efficiency is run by bull by month. Next comes the sire efficiency by breed by month. Thus it is possible to see how any one bull is doing in any one day or month, and breed efficiency is also available for the month.

"The same cards used to make up the sire analysis are now sorted again by technician number. These cards are then taken to the



## BARN BREEDING RECORD

**TACK**

# BARN BREEDING RECORD

[illegible]

### SIRE OR LOCAL EFFICIENCY RECORD

Year

[illegible]

Figure 82. Record keeping is essential to an efficient artificial breeding program. This sire or local efficiency record form is used by the Northeastern Pennsylvania Breeding Cooperative, Tunkhannock, which has achieved top conception rates in the herds of its members. The word "local" can mean a county or a subsection or any other type of affiliate of the organization which dispenses semen. The word "drawing" is synonymous with "collecting," the term more widely used.

tabulator, and technician efficiency is run. This report tells us how each technician is doing each month.

"At the end of each year the cards for the entire year are run through the accounting machine, and a summary card is punched automatically on the reproducing punch giving the member's account no. and total number of first services for each year. The summary punched cards are used to make out the member's dividend equity checks or to send out notices of advice telling members the amount of dividend held for them during the last fiscal year.

"The name and address cards used in preparing statements are also used to make labels for publications or any type of special mailing going out to all members. Labels are also run on the IBM machine for all semen packages shipped from the association.

"Many other statistical or accounting chores can be done with the type of machine described."

#### TECHNICIAN TRAINING

Sometimes the highly important program of training technicians is conducted as a short course by a state college of agriculture. In other cases it is supervised by the manager with the aid of the most experienced of the technical staff of the central breeding organization. The following training schedule reported by Albretsen (1958) is representative of those usually given in various parts of the United States from one to four times per year, depending on the need. Cornell University was one of the pioneers in inaugurating this highly essential type of educational program.

#### SCHEDULE OF TRAINING COURSE FOR ARTIFICIAL INSEMINATION TECHNICIANS

(Given at Cornell University)

##### *Monday*

##### Morning

9:00-10:00

Enrollment

10:00-11:00

The development of artificial insemination

11:00-12:00

Attachment of sleeve and glove

##### Afternoon

1:00- 2:30

Anatomy of the reproductive tract of the cow

2:30- 4:30

Functions of the female reproductive tract

SCHEDULE OF TRAINING COURSE FOR  
ARTIFICIAL INSEMINATION TECHNICIANS (*Continued*)

*Tuesday*

Morning

8 30-12 00     Bull reproductive physiology, bull semen—its production, characteristics, dilution, and storage (Breeding Lab )

Afternoon

1 00- 2 30     Equipment used and procedures followed in insemination at the farm

2 30- 3 30     Insemination demonstration

3 30- 5 00     Practice with cow reproductive tracts

*Wednesday*

Morning

8 00-12 00     Insemination practice

Afternoon

1 00- 1 30     Examination of technicians (Quiz 1)

1 30- 3 00     Milk Production Records and their relation to the A B program

3 00- 4 30     The problem of sterility and disease in cattle

*Thursday*

Morning

8 00-12 00     Insemination practice

Afternoon

1 00- 3 00     Principles of livestock improvement through breeding

3 00- 4 00     Tour of Dairy Records Processing Center

*Friday*

Morning

8 00-12 00     Insemination practice

Afternoon

1 00- 1 30     Examination of technicians (Quiz 2)

1 30- 3 00     Finances, rules and regulations

3 00- 5 00     Breeding receipts, herd books, and non returns (conception rate)

*Saturday*

Morning

9 00-12 00     Type appraisal demonstration and discussion

*Monday*

Morning

9 00-12 00     Insemination practice

## Afternoon

- 1:00- 2:00 Timing and other management factors related to breeding (optimum time to inseminate)  
 2:00- 3:00 Developing good membership and public relations  
 3:00- 5:00 Inheritance of production

*Tuesday*

## Morning

- 8:00-12:00 Insemination practice

## Afternoon

- 1:00- 3:00 Artificial insemination production results  
 3:00- 5:00 Discussion of research projects

*Wednesday*

## Morning

- 8:00-12:00 Insemination practice

## Afternoon

- 1:15- 4:30 Laboratory on financial records

*Thursday*

## Morning

- 8:00-12:00 Insemination practice

## Afternoon

- 1:00- 1:30 Examination of technicians (Quiz 3)  
 1:30- 2:00 Using frozen semen in the field  
 2:00- 2:30 Ear-tagging (cow identification)  
 2:30- 4:00 Type and production standards for sires

*Friday*

## Morning

- 8:00-12:00 Insemination practice

## Afternoon

- 1:00- 3:00 Final Examination  
 3:00- 3:30 Picture taken  
 6:00- 8:00 Banquet

*Saturday*

## Morning

- 9:00-10:00 Review of final exam  
 10:00-11:00 You and the Artificial Insemination Program  
 11:00-12:00 Discussion of apprentice training

## ANNUAL MEETINGS AND REPORTS

Among the notable characteristics of progress making breeding groups are well attended annual meetings. These stem from a program agenda that is meaningful and attention getting in makeup, and widely publicized well in advance. It is the occasion to recount both achievements and obstacles, rewards and penalties. With few exceptions, the period since 1938 has been one of advancement in many countries for most of the organizations, once they began to draw in their lines, avoid some of the duplication of effort and create larger, more efficient operating units. Fairly typical of the reports given by managers are the following excerpts from the one by Marshall C. Carpenter (1958) of the Kentucky Artificial Breeding Association Inc.

Your association can again say "the best year ever." This has been a constant statement for most of the years of our history. However, this year marks the most accelerated rate of growth since 1951. We bred 89,300 cows the past year, which is a 13.3 per cent increase over last year's 78,800. I know it wouldn't have happened if service had been poor or AB daughters were unsatisfactory.

I would like to summarize briefly the year's happenings with some of my interpretations.

All breeds increased in service numbers. The use of the beef breed may be surprising, but this is following the national trend and demonstrates how an industry can render more services than those originally planned.

*Services per Bull Higher* Services per bull averaged 2,320 compared to 1,980 last year. This is a big increase and it puts us near the national average.

Eight of our bulls were bred to 45,000 cows or over half the total. One proved bull was bred to 11,329 cows for a new high yearly total for our association.

Breeding efficiency has had its greatest improvement this year. It averaged 71.4 per cent on 60 to 90 day non-returns for the first ten months that I can report at this time. This is a 3.8 per cent improvement over last year and it can be credited to research done by Pennsylvania State University.

One interesting observation is our Hereford bulls. They averaged 77.4 per cent or 6 per cent more than our dairy breeds. This is hard to understand but seems to be similar to other states.

We have been fortunate to have our bulls stay in service longer than the three-year average of the United States in artificial breeding. Our depreciation figures of 20 to 25 per cent have been reliable. Seven new bulls were added this year.

As artificial breeding increases, the number of good association-proved sires available increases. The studs are tending to prove more young bulls.

The locals have remained constant as to the number operating in Kentucky. There has been a continued trend to have more full-time men. We have 11 locals using two full-time men, and practically all have a relief man.

I made a study of the years' experience of our technicians. The ones working now have a total of 291 years' work, or an average of four years and eight months service. You can see the average member is getting service of an experienced man. One technician has now bred over 36,000 cows.

*New Semen Diluter.* New methods have been adopted this year that have been very important in improving service for members.

The milk glycerated semen has increased breeding efficiency and also the time semen can be used. It has made semen useable for four or five days with results equal or better than two days' use previously. A study of this process shows a breeding efficiency of 72.9 per cent on 51,800 services. It was 67.8 per cent on the 22,800 services by the old process.

The bull power studies were a great aid to our industry. They show that many bulls can be used twice weekly. The total services or sperm production is increased by 67 per cent by this process.

Seven bulls are now on this twice weekly collection, making their service available every day and at no more cost. It is possible to collect, process, and ship enough semen from one bull to breed 80,000 cows in one year. I make this statement based on present procedures used and the knowledge that one bull can produce 800 billion sperm yearly.

Summarizing, this year shows a great rate of gain in service number. The goal for next year is 100,000 cows. One month of year is complete with an increase that indicates we can do it. We must realize it takes planning and good service. It is too easy to relax and expect increases will naturally follow.

We have the best average group of technicians in our history. Local boards are helping more in promotion and public relations. All these factors added together can insure success for the vital service contact at the local level.

The following excerpts are from a report by Max Drake of the Northern Ohio Breeding Association in 1959. Mr. Drake is another manager with twenty years of experience. His statements likewise reflect an abiding enthusiasm for the artificial insemination project and an intelligent interest in the further improvement of techniques and methods of operation as a means of increasing members' benefits.

We have been on a 100 per cent frozen semen program for 18 months and think it is the most important development in artificial breeding since its inception. The conversion from liquid to frozen semen was made without loss of conception rate. As a result we have been furnishing the semen of any bull at any time to any member or patron.

This by necessity in an organization as large as ours, means that we had to adopt a pricing policy consistent to keep supply and demand in line, in order to make such a choice of bull program feasible. No bull in our stud can be stretched to meet the demands if he happens to be unusually popular and at the same time a bull of good conception rate. As a result we have bulls at \$6 \$7 \$8 and \$10 fees to our own members, and it is a program that sells itself very well. I anticipate that as time goes on and we grow in size we will probably have greater differences in bull evaluations or at least service fees different from these here quoted. I can very easily see where a real top sire might be priced to our members at considerably above \$10. Actually, I know of no other way to ration such a bull on a fair and equitable basis.

Our association is now equipped to do a job with frozen semen. Admitting the merits of one or two of the best liquid semen programs now operating we would not now be interested in returning to such. Our next step would have to be one of very considerable improvement, conceivably drying, etc.

Some real answers are needed on semen quality. We have come to the conclusion after studying most of the records of our recent services on an ejaculate basis that the biggest difference between good bulls and medium or poor bulls on conception rate is not an actual bull difference as such, but that the difference lies largely in the proportion of good and poor ejaculates that the various bulls throw. For instance in studying several of our top Holstein bulls we found that they were consistently running conception in the 70 per cent range week after week on an ejaculate basis. But then something would go wrong and we would find one of these ejaculates dropping from the 70's down into the 30's or 40's then bouncing right back again. Now obviously, there was some measurable difference in that semen and we didn't catch it, nor do I believe

that any of the present methods of semen evaluation would catch it. At the same time, I remember a particular Jersey bull, whose conception was always below the average for that breed, and yet, about a third of his ejaculates were rated in the 70 to 80 per cent conception. This means, that if we had some method of accurately identifying those medium to poor ejaculates and discarding them he would have been one of the good conception bulls in our stud. Our semen quality determinations are based on the use of the microscope and the photometer.

Our normal procedure of breeding cows hasn't varied a great deal over the years. We still try to inseminate the "in heat" A.M. cows sometime during the day and, normally, to try to care for the cows of the previous P.M. and evening the first thing in the morning. We have some men who do cover their territory twice per day, and it is possible in my mind that we may be reaching some of these cows too soon.

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## Frozen Semen

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H A HERMAN

When we speak of frozen semen, we refer to semen frozen and preserved at  $-79^{\circ}\text{C}$ , utilizing dry ice and alcohol as refrigerant, or semen preserved at  $-196^{\circ}\text{C}$  in liquid nitrogen containers. Both methods are in common use in the United States. Many breeding organizations in Europe use dry ice and alcohol to provide a storage medium, and many also use liquid air, which, like liquid nitrogen, can provide storage at around  $-190^{\circ}\text{C}$ . Liquid  $\text{CO}_2$  also may be used.

### ADVANTAGES OF FROZEN SEMEN

The use of frozen semen is one of the spectacular developments in modern day artificial insemination programs. The artificial insemination program for cattle in particular, has been greatly enhanced by the development of frozen semen. Its advantages are many, and because of the costs involved in maintaining fresh semen at all times, frozen semen is considered a most important adjunct to liquid semen for many organizations. Some organizations, because of their location, service needs, and shipping problems, have installed a 100 per cent frozen semen program with satisfactory results. This trend will continue.

The advantages of frozen semen are

1. With frozen semen selective mating is possible. A dairyman can use the sire of his choice at any time.

2. Frozen semen is valuable in carrying on the influence of sires long after they are gone. Semen stored in the frozen state for three to over four years has successfully been used and living calves produced.

3. Frozen semen permits almost 100 per cent utilization of the semen from a given sire. No semen need be discarded because of age, as is true with liquid semen.

4. Transportation costs of semen from the bull stud to area technicians is greatly reduced. Instead of daily or every-other-day shipments of liquid semen, the technician may have his supply of semen from selected bulls replenished every two or three weeks. Transportation of frozen semen is by truck, railway express, and parcel post. The cost of delivery of semen cannot be taken lightly; it is a major expense for most organizations. Full-fledged frozen semen organizations deliver frozen semen by truck on regular schedule. It is a growing program.

5. Selected matings to sires in widely located areas are possible. It is even possible to make matings to bulls on the Channel Islands, Holland, and England with cows in the United States. Shipment from coast to coast and throughout the world is now common practice.

6. Disease control, especially with respect to frozen semen, has been a point of question. It is known that freezing does not kill *Vibrio fetus* organisms, but Dr. W. N. Plastringe, of the Connecticut Experiment Station, and Dr. H. L. Gilman, of Cornell University, in exhaustive trials with virgin heifers have been unable to demonstrate *Vibrio* transmission in a single instance. It must be realized that bulls in general artificial insemination use are carefully checked by periodic examinations for *Vibrio fetus*, trichomoniasis, and general venereal diseases. Infected bulls are not employed.

#### HISTORY OF FROZEN SEMEN

Davenport recorded in 1897 that human spermatozoa would survive freezing at  $-17^{\circ}\text{C}$ . This observation received little attention, and it was not until 1938 that further work was reported. At this time Luyet and Hodapp (1938) found that a large percentage of frog sperm showed motility after being frozen. They first partially dehydrated the sperm in 2M sucrose, then immersed it in liquid air,



Figure 83. Frozen semen was used to inseminate the dam of this Holstein heifer, a third generation artificial insemination product. When she was born, in 1958, her sire had been dead over four years. (Courtesy New York Artificial Breeding Cooperative)

and later thawed it rapidly by plunging the semen into a medium warmed to  $20^{\circ}\text{C}$ . This technique was based on instantaneous freezing in order to secure vitrification rather than crystallization of the intracellular water.

Another worker, F. Jahnel (1938), reported some success in reviving human sperm which had been frozen in glass tubes for 40 days at  $-79^{\circ}\text{C}$ , and also some kept for shorter periods at  $-196^{\circ}\text{C}$ . and  $-269^{\circ}\text{C}$ . He apparently did not take any precautions to insure utmost rapidity of freezing and thawing.

A pertinent literature review by Luyet and Gehenio appeared in 1940. It contains information on the ability of a variety of organisms to survive exposures to very low temperatures. Luyet reports that the preservation of life at low temperatures depends on preventing the formation of intracellular ice crystals, either by dehydrating the cells before freezing or by very rapid cooling and rewarming.

Shettles (1940) attempted to revive human semen after it had

been frozen to temperatures of  $-79^{\circ}\text{C}$ .,  $-196^{\circ}\text{C}$ ., and  $-269^{\circ}\text{C}$ .. He obtained a low percentage of motile sperm, and emphasized that semen should be as fresh as possible at the time of freezing.

Shaffner *et al.* (1941) froze fowl sperm to  $-70^{\circ}\text{C}$ . after partially dehydrating it and adding levulose. About one-third of the sperm could be revived.

Hoagland and Pincus (1942) used a variety of plasmolyzing solutions. Freezing was accomplished in liquid nitrogen at  $-195^{\circ}\text{C}$ ., and thawing by plunging into a medium at  $35^{\circ}\text{C}$ . Better results were obtained with human than rat, moose, guinea pig, rabbit, or bull sperm. However, the revival rate was poor.

True storage of frozen semen was investigated in 1945 by Parkes at the National Institute for Medical Research, Hampstead, England. He noted that previous unsuccessful attempts had been made with the semen in thin films or small-bore capillary tubing, and so he designed an experiment to compare the freezing of human semen in small-diameter capillary tubes with the freezing of semen in larger tubes. Successful results were obtained at  $-79^{\circ}\text{C}$ . and  $-196^{\circ}\text{C}$ . with the larger tubing, but not with the smaller. Parkes was able to freeze human semen in ampoules and maintain them for two to eight days at  $-79^{\circ}\text{C}$ . An abundance of spermatozoa survived when thawed. No record of fertility of this semen was obtained.

In 1949 the English workers, Polge, Smith, and Parkes, reported that glycerol-containing diluents made possible the complete revival of motility in fowl spermatozoa kept at  $-79^{\circ}\text{C}$ . for long periods. They also state that glycerol assists the survival of human sperm.

Smith and Polge (1950b) found glycerol to be outstanding among polyhydric alcohols and their derivatives in protecting spermatozoa against low temperatures. These workers indicate that glycerol modifies the process of ice crystal formation and dissolution in the medium, so that the damage due to pressure and other mechanical effects is reduced. Glycerol was found to have a deleterious effect on the fertilizing capacity of fowl sperm, but this has not been found true for other species. Cattle semen is one of the notable exceptions.

Glycerol permits safe freezing. Smith and Polge (1950a) investigated the storage of bull and goat spermatozoa at low temperatures. Ten per cent and 15 per cent glycerol-sodium citrate buffered bull semen mixtures cooled slowly from  $2^{\circ}\text{C}$ . to  $-79^{\circ}\text{C}$ . and then thawed

showed 50 to 90 per cent survival. Ninety per cent revivals were obtained in samples containing 15 per cent glycerol and cooled in 14 stages of two and one half minutes each. Storage at 2° C after thawing resulted in a reduction of motility after 24 hours as compared with unfrozen controls. Goat spermatozoa behave similarly.

The first real success with bull semen was reported by Stewart in 1951. One calf was produced among many attempts at fertilization using frozen semen.

The effects of glycerol on unfrozen bull semen were investigated by Polge and Rowson (1952), and they found that it did not diminish fertilizing capacity. In fact, the pregnancy rate of semen containing 15 per cent glycerol was 76 per cent, whereas similar semen without glycerol gave a rate of 50 per cent.

#### FREEZING SEMEN

The method of semen freezing as adapted by the British workers is described by C. Polge, London, this is essentially the method followed by most American workers to date.

I Immediately after collection, dilute the semen with a diluent made up of 50 per cent egg yolk and 50 per cent by volume of 2.94 per cent sodium citrate (dihydrate) solution. Usual dilution rate—1:1 at normal room temperature.

II Cool diluted semen in refrigerator for three to five hours. Temperature 5° C.

III Dilute 1:1 with 16 per cent glycerol by volume in 2.94 per cent sodium citrate solution. Temperature 5° C. Add 0.5 mg of streptomycin sulfate per ml. Final concentration is then 8 per cent glycerol, 25 per cent egg yolk, 2.2 per cent sodium citrate solution. (Some workers add glycerol in 5-3-1 portions over a period of several hours. It is not clear how advantageous if at all this procedure may be.)

IV Equilibration period of 6 to 20 hours at 5° C. Most workers prefer 16 to 20 hours. Best results seem to indicate a 16 to 20-hour equilibration period.

V Package semen in vials or ampoules for freezing. Label properly.

VI A. Freeze—using dry ice and alcohol (or acetone) at rate of 1° C per minute from 0° C to -15° C. This is a critical stage in the process, and the timing must be accurate. Caution—The whole secret of cell freezing is to prevent crystallization of water in the cells and to freeze rapidly, with glycerol acting as a protective agent, to the vitreous state.

B. Freeze from  $-15^{\circ}\text{C}$ . to  $-70^{\circ}\text{C}$ . at the rate of  $-3^{\circ}$  to  $4^{\circ}\text{C}$ . per minute.

VII. Store at  $-79^{\circ}\text{C}$ . or below. The storage temperature is important. Cells cannot be warmed and refrozen. A temperature rise above  $-65^{\circ}\text{C}$ . renders cells less fertile. Continuous storage temperatures of  $-70^{\circ}$  to  $-79^{\circ}\text{C}$ . or below are necessary.

VIII. To use frozen semen—Place the ampoule of frozen semen in water at  $5^{\circ}\text{C}$ . for a few minutes. Then use in the usual manner. Many technicians carry a container of water and ice cubes so as to thaw the semen between farms. This is possible where the sire to be used has been designated.

The above procedure for processing, freezing, and storing semen is standard technique, but at the same time modifications are made by many operators. The percentage of glycerin used may vary from 7 to 12 per cent by volume, the equilibration time from 6 to 20 hours, and the percentage of egg yolk from 20 to 25, with apparently equally successful results.

Persons expecting to freeze semen should study the procedure carefully and adopt the operation best adapted to their needs. The references in this field are voluminous, but some of the most pertinent are listed at the end of this chapter.

#### EXTENDERS FOR FROZEN SEMEN

Egg yolk-citrate extender or milk extender (diluter) may be used for frozen semen with equal satisfaction.

New Jersey Agricultural Experiment Station Circular 573, issued in 1955, outlines the steps for preparing each type of extender, and these are repeated here:

I. *Egg Yolk Diluter Preparation (Solution A)*. Transfer the yolks of four fresh eggs into a 250-ml. graduated cylinder; add an equal volume of sterile, distilled water; mix thoroughly, and allow to stand for a few minutes.

(*Solution B*). To a 100-ml. graduated cylinder, add 40 ml. of solution A, 2 gm. of sodium citrate dihydrate, 100 mg. of streptomycin sulfate; and make up to a total of 100 ml. with sterile distilled water. Mix thoroughly.

(*Solution C*). To a 100-ml. graduated cylinder, add 14 ml. of glycerol, 40 ml. of solution A, 2 gm. of sodium citrate dihydrate; and

make up to a total of 100 ml with sterile distilled water Mix thoroughly

By combining equal quantities of solutions B and C, a diluter of the following final composition will be formed 20 per cent egg yolk, 2 per cent sodium citrate dihydrate, 7 per cent glycerol, and 0.5 mg of streptomycin sulfate per ml

**II Milk Diluter Preparation (Solution A)** Heat whole, homogenized, pasteurized milk in a double boiler to 92° to 96° C for ten minutes and cool

(Solution B) To each 100 ml of solution A, add 100 mg of streptomycin sulfate

(Solution C) To a 100-ml graduated cylinder, add 16 ml of glycerol and 84 ml of solution A, mix thoroughly

By combining equal quantities of solutions B and C, a diluter of the following final composition will be formed 92 per cent heated, whole, homogenized milk, 8 per cent glycerol, and 0.5 mg of streptomycin sulfate per ml

#### PROCESSING AND FREEZING EQUIPMENT

The equipment for processing semen can be purchased from the various suppliers of materials used in the artificial insemination field and laboratory supply houses Advertisements of same are found in *The A I Digest* Information may also be obtained from the nearest agricultural experiment station or artificial breeding organization

The usual freezing equipment consists of a cooling bath, equipped with wire rack to maintain semen in place in flasks during the equilibration period, flasks and graduated cylinders for measuring materials in formulation of the extender, glycerol, and semen, ampoules made of glass, usually 1.0 ml to 1.2 ml, for packaging the pre-cooled extended semen into suitable doses for insemination

Ampoules must be permanently labeled and carry name and registered number of the sire A glass marking ink may be used, or supplies of ampoules may be permanently labeled There are marking machines on the market that will do this job satisfactorily for large operators

The ampoules are sealed with an oxygen gas burner, or by means of a sealing machine This equipment is easily procurable

Wire racks are necessary to hold the ampoules during freezing. These racks should be the same size to fit into permanent storage facilities.

The semen in the ampoules is frozen by placing the racks in a bath of isopropyl alcohol, or acetone, and adding dry ice a bit at a time to control the freezing rate. As mentioned, the temperature is lowered about  $1^{\circ}\text{C}$  per minute between  $5^{\circ}\text{C}$  and  $15^{\circ}\text{C}$ , and  $3^{\circ}$  to  $4^{\circ}\text{C}$  per minute between  $-15^{\circ}\text{C}$  and  $-79^{\circ}\text{C}$ .

A mechanical agitator assembly, a dial thermometer, range  $-100^{\circ}\text{C}$  to  $+40^{\circ}\text{C}$ , and a clock to regulate timing will greatly facilitate the freezing procedure and aid in uniform results.

After freezing, the ampoules in their racks are transferred immediately to storage facilities. The temperature is maintained at  $-79^{\circ}\text{C}$  or below at all times until the semen is used for insemination.

#### FROZEN SEMEN REFRIGERATION

**Dry Ice and Alcohol.** For most storage of frozen semen, dry ice and alcohol, which will maintain a maximum low temperature of  $-79^{\circ}\text{C}$ , are employed most widely today. However, the use of liquid nitrogen, liquid air, or liquid carbon dioxide, or mechanical refrigeration, is becoming more prevalent. No doubt there will be many changes in equipment as our experience increases in this field.

The advantages of dry ice and alcohol as a refrigerant are that the procedure is simple, equipment is fairly cheap, and storage containers come in various sizes and can thus fit many operations in the field.

Disadvantages are that a constant supply of dry ice must be available. This is not always possible—particularly in isolated areas and some foreign lands. It is also necessary to keep a constant check on the dry ice and keep the storage equipment properly supplied, or the entire batch of semen may be lost.

**Vacuum Jar.** For occasional matings, or in order to keep a small supply of frozen semen on hand for immediate herd use, the herd owner will find the one-gallon, rack-fitted, vacuum jar satisfactory and economical (see Figure 84). The jar must be kept well supplied with dry ice and alcohol. As a rule, such jars need to be re-iced about every three or four days.





Figure 84 Vacuum jar for carrying or storing small quantities of frozen semen  
(Unsem kit Co., Baraboo, Wisconsin)

**Dry Ice Storage Chests** Chests like the one illustrated in Figure 85, are available in various sizes from most supply houses

Semen is removed from these containers by means of forceps so as to prevent warming contents of the entire container

**Mechanical Refrigeration** Mechanical refrigeration is used as an alternate for dry ice. Electric refrigerators are available in 1-cubic foot to 54-cubic foot sizes, the largest will hold 57,600 ampoules of frozen semen



Figure 85 This frozen semen chest made of polystyrene will hold 1,200 ampoules. It requires 50 pounds of dry ice and 5 gallons of alcohol.

The larger mechanical refrigerators are used for central storage, usually at the headquarters of an artificial insemination organization. The smaller mechanical units, 2 to 5 cubic foot capacity, are used to supply technicians, often several, operating out of a field office. The smaller units, and likewise dry ice and alcohol storage units, are replenished with semen from the central storage.

Large mechanical and large dry ice alcohol storage chests are used for "custom" storage. If a power failure occurs, dry ice is often used to supplement a mechanical box. Some organizations maintain an auxiliary Diesel power unit to supply electricity under emergency conditions.

**Liquid Nitrogen.** Liquid nitrogen is the fourth coldest substance known. At atmospheric pressure it has a boiling point of  $-196^{\circ}\text{C}$  ( $-320^{\circ}\text{F}$ ).

Growing evidence indicates that temperatures below  $-79^{\circ}\text{C}$  ( $110^{\circ}\text{F}$ ), which is the physical limit of dry ice and alcohol, may be most desirable for semen storage.

**Dry Ice Versus Liquid Nitrogen.** American Breeders Service, Chicago, Illinois, the largest artificial insemination organization in the



Figure 86 Removing a tray of frozen semen from the 54-cubic foot electrically operated storage unit at the Central Ohio Breeding Association Columbus. This unit holds over 57 000 ampoules of semen. Dual refrigeration machinery assures safe temperature at all times.

world has conducted rather exhaustive research on this point and in 1957-1958 installed a 100 per cent frozen semen program involving some 1 100 technicians and 1.2 million cows in the United States and abroad. Liquid nitrogen and appropriate containers were introduced and are giving good results. That organization reports 71.5 per cent on a 60 to 90-day non return basis for over 1 million cows on a first service basis.

## Frozen Semen

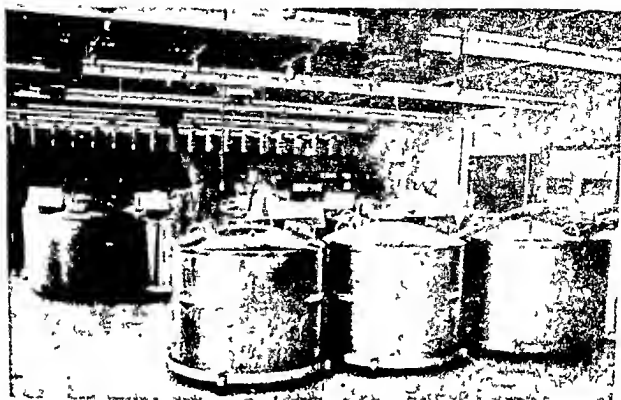


Figure 87. Liquid nitrogen shipping and storing containers at Americon Breeders Service, Madison, Wisconsin. Electronic recorders in each container assure enough liquid nitrogen to maintain the 40,000 ampoules in good condition until arrival at their destination as much as two months later.

Larson and Graham (1958) studied 75-day first service non-returns on about 1,000 cows on the artificial insemination program in Minnesota and found that dry ice-alcohol storage ( $-79^{\circ}\text{C}.$ ) gave 69.3 per cent non-returns, and split samples of semen stored by liquid nitrogen ( $-196^{\circ}\text{C}.$ ) gave 71.9 per cent. They did not consider the results statistically significant. Pickett *et al.* (1959) in Connecticut conducted an investigation employing the "split sample" technique to test semen preserved with liquid nitrogen. They used liquid, but glycerolated semen for the control animals. On a 60 to 90-day non-return basis, they found that the non-return percentage was 77.7 for 332 cows inseminated with liquid semen. On 329 cows inseminated with frozen semen stored in liquid nitrogen containers the non-return percentage was 72.9. The authors did not consider the non-return differences between the two methods significant and that either method provided satisfactory storage.

Liquid nitrogen storage drums for frozen semen may now be obtained from several concerns, and in the future more revealing in-

formation on the advantage of extremely low temperatures should be forthcoming

**Liquid Air.** Many European workers are using liquid air as a refrigerant for frozen semen. The process is adequately described by B. R. van Wulften Palthe (1959). Liquid air provides a holding temperature below  $-150^{\circ}\text{C}$ , that is, temperatures comparable to those of liquid nitrogen, moreover, fairly trouble free equipment can be used, and dry ice is not needed for central storage on a long time basis.

**Liquid Carbon Dioxide.** Equipment utilizing liquid carbon dioxide as the refrigerant for frozen semen is available. The temperatures are as low as those for dry ice, or may be lower under pressure. An important practical advantage of this equipment is that it is free of mechanical problems. Storage chests utilizing liquid carbon dioxide are available in sizes that will store 100,000 ampoules of semen. Because of its simplicity, freedom from mechanical failures, and economy of operation, liquid carbon dioxide chests may play an important role in artificial insemination operations in the future.

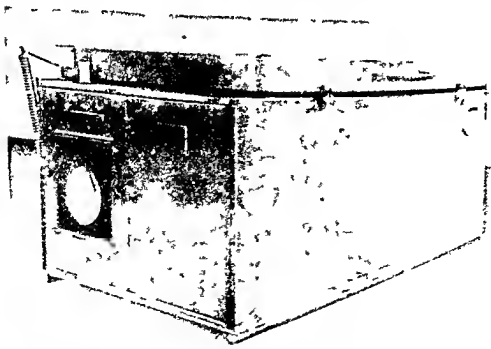


Figure 88 A liquid carbon dioxide storage cabinet that holds 100,000 ampoules of semen.

## EFFICIENCY OF FROZEN SEMEN

Polge and Rowson (1952) have reported their results with frozen semen in the insemination of 285 cows. The conception rate (three-month non-return) was 66.3 per cent. Semen stored for as long as 32 weeks was just as good as fresher frozen semen. Inseminations were made with samples diluted up to 1:40 and kept at  $-79^{\circ}\text{C}$ . There was no indication of impaired fertility in any of the dilutions.

Frozen semen usage in the United States indicates probably 2 to 5 per cent lower non-returns than fresh semen. This figure varies greatly with the selection of semen samples and bulls by breeding organizations.

A number of organizations in Canada and the United States have been able to achieve equal results with frozen and liquid semen.

## USE OF FROZEN SEMEN

During 1958, some 51 of the 71 bull studs in the United States utilized frozen semen. Ten bull studs used frozen semen 100 per cent.

Forty-four of 71 bull studs were freezing and storing semen for cooperating breeders on a custom basis.

Inventories made by The Holstein-Friesian Association of America, Brattleboro, Vermont, which has 47 per cent of its registration from artificial insemination service, showed that in 1958:

1. 608,994 ampoules of semen from 780 bulls were in storage by some 51 bull studs.

2. 522,560 ampoules of semen were in storage as a product of 509 live bulls; there were, however, 57,808 ampoules from 116 bulls that were dead or had gone out of use.

3. Several calves—Ohio, New York—had been born four years after the sire in question was dead. Semen in England has been used after four years of storage.

There does seem to be a slight decline in fertility of frozen semen as the length of storage time increases. The drop in fertility seems to occur during the first few weeks in storage and declines little thereafter. The rate at which frozen semen declines in fertility needs further research and will be determined in the future. Many ex-

periment stations, some on projects supported by the National Association of Artificial Breeders, are now working on this problem

#### CUSTOM FREEZING OF SEMEN

Many artificial breeding organizations are offering cattle breeders in their vicinity "custom freezing" service "Custom freezing" offers any owner of an outstanding bull the opportunity to use such a bull for artificial breeding, even after he is dead

Organizations that practice "custom freezing" will go to the farm, collect semen from a bull, evaluate the semen, freeze same if satisfactory, store the semen, and provide the owner with a supply as needed

This seems a logical step, and a service that any purebred owner should utilize The artificial breeding organizations have the equipment the storage facilities, and experience in the technique of freezing semen Furthermore, the purebred breeder often can sell semen from outstanding bulls through organized artificial breeding organizations This results in selected matings and satisfactory service This type of service is on the increase

As a rule, purebred breeders who offer services from outstanding bulls will fare better by offering their bulls through the organized artificial breeding organizations These organizations are equipped to do a complete job and will relieve the herd owner of the task of dispensing semen in a competitive market

Custom freezing service is available in nearly every part of the United States All a herd owner needs to do is get in touch with his nearest artificial breeding organization if he desires frozen semen service At the present time, over 50 bull studs are freezing semen on a custom basis, and there are at least three private operators who specialize in collection, freezing, and storage

**Freeze While Bull Is Healthy** If a bull is deemed worthy of inclusion in the frozen semen program, it is wise to have the semen collected and frozen while the bull is healthy and active Too many breeders wait until a bull is crippled or waning in fertility before arranging to have his semen frozen Freezing will never improve semen and semen from bulls of low fertility will appear worse It is a lesson we must learn

## DISADVANTAGES OF FROZEN SEMEN

The artificial insemination industry looks upon frozen semen as a very useful tool, which, if used intelligently, can perform miracles in breeding. There are a few disadvantages, however, that need to be recognized and proper safeguards exercised.

Frozen semen may limit the number of bulls used. There is no "sure fire" method of selecting bulls for herd improvement except the *progeny test*. This is the program the artificial insemination industry seeks to follow. "Get a good proved bull and use him heavily." Pages upon pages of material have been written by students of breeding on how to select bulls. This endeavor is commendable, but in terms of realistic values, we are still subject to the laws of Mendel. Most selection must be based on previous performance; and sometimes we do not know the selection intensity—and even these values are questionable in many cases. Here is why the frozen semen program needs careful screening so that the tremendous advantages it affords can be properly utilized:

1. Some bulls, about one-third, produce semen that will not undergo the rigors of freezing. Thus, unless we are very careful, we may wind up selecting bulls largely on a "fertility basis" and ignore some of the better production bulls that may be lower in fertility! *Kanowa King Posch Neptune*, shown in Figure 18, is typical of the bulls that could be used very heavily. We believe fertility is important and should be stressed. However, in the present-day competitive program in artificial insemination which really demands "get the cow settled," some very great bulls may be lightly used. It is a case requiring wisdom and careful judgment.

2. Frozen semen is expensive! The cost of dry ice and maintenance of a semen bank is a daily expense. Organizations that have both a liquid and a frozen semen program find their overhead costs increased because of frozen semen. Yet, we subscribe to the idea that frozen semen is the ideal answer for people who want selected matings. At present they represent about 16 to 15 per cent of the dairymen who use artificial insemination service.

3. Heavy utilization of frozen semen limits the number of sires used. It is possible for one sire to have over 100,000 offspring. One organization reports four bulls, two still in active service, that have



over 100,000 first services. Several other sires have achieved this distinction. There is no argument as to the wisdom of heavy use of any bull of greatness so that he can sire many progeny. The only question is "*What sire, or sires should be entrusted with the major inheritance of any man's herd?*" If our selection methods were perfect, there would be no argument. As the picture stands, we would advise against the use of too few sires. However, when truly great bulls are found, even by our present methods of appraisal, their heavy use can add much to the genetic "bank account" for cattle.

#### REGULATIONS FOR PUREBRED DAIRY CATTLE

The regulations for fresh semen, as set by The Purebred Dairy Cattle Association of the United States, place the burden of responsibility for accuracy and honesty on the producing organization, and these apply equally to *frozen semen*.

In January, 1954, representatives of The Purebred Dairy Cattle Association and the National Association of Artificial Breeders drew up the regulations for frozen semen now in effect. They read as follows:

1. When frozen semen is used, the letters "FS" are to be written in the lower right hand corner of the breeding receipt.
2. Semen Producing Businesses freezing semen must keep an inventory of frozen semen on hand and this inventory available at all times as are their other records.
3. Upon the disposition of a sire, either by death or sale, Semen Producing Businesses must report the number of ampules of frozen semen on hand to the breed registry organization involved.
4. Semen Producing Businesses performing the service of freezing semen from sires not owned by the Semen Producing Business must report *monthly* the number of ampules frozen, giving the name and number of the sire and the name and address of the owner.
5. Where 10 or more ampules of semen are sold by one breeder to another, a breed association transfer and the necessary transfer fee are required. Each breed association furnishes the necessary "Transfer Forms."
6. Every bull used for breeding of purebred cattle and involving frozen semen must be blood typed.

## BEEF CATTLE AND FROZEN SEMEN

Frozen semen is already playing an important role in beef cattle breeding. Although beef cattle regulations for registration of purebreds prohibit the use of semen from organized breeding studs but do allow three or four persons to own a sire jointly, the owners of such a sire may use frozen semen in their own herds whether they are neighbors or live in distant states.

In 1957, beef semen was utilized in about 11 per cent of all artificial inseminations of dairy cattle. This trend will increase as long as beef prices are above the milk-feed rates for below-average dairy cows. These were largely commercial cattle matings.

By using frozen semen, the purebred beef producer can utilize an outstanding sire in his own herd and still share the sire with a friend, counties—or states—away. It is being done every day!

Beef cattle producers have indicated much interest in "a better calf crop," control of breeding troubles, Performance Registry, and "Progeny Tested Sires." The latter are beef bulls whose progeny have been recorded for rate of gain.

This trend will probably continue. Purebred beef cattle breeders want the very best sires; the commercial producer wants efficient gain. We believe this trend will continue as more beef producers interest themselves in efficiency of gain. There are roughly two beef animals for every dairy animal in the United States. Thus, there is opportunity for a vast new program of cattle improvement.

## DISTRIBUTION OF FROZEN SEMEN

It is a foregone conclusion that shipping costs can be materially reduced by the use of frozen semen. This is particularly true if truck transportation is used and replenishment of supplies is necessary only every few weeks. Many artificial insemination organizations do deliver frozen semen by truck. American Breeders Service, for example, has a fleet of trucks covering the United States, not only to deliver semen but to replenish liquid nitrogen in technicians' storage facilities.

## FOREIGN SHIPMENTS OF FROZEN SEMEN

Shipment of frozen semen from the United States to Central America, South America, Mexico, Cuba, and other countries is well established. For the most part, shipments today are to private herd owners. These countries do not have many large-scale artificial breeding organizations, however, a few of the small breeding operations are utilizing frozen semen with excellent results. The trend will grow.

It is logical that the United States should furnish the Latin American countries with semen from top quality bulls, and the frozen semen program can easily do the job. Expensive bulls imported from the United States, Canada, and Europe into South and Central America often have a difficult time in becoming acclimated. Many are victims of anaplasmosis and local diseases of cattle. In some cases, the fertility of semen of the imported sires is greatly reduced by submarginal ailments. If frozen semen, rather than the valuable sire, can be imported from the North American continent or Europe, crosses with native cattle can be achieved just as successfully. The frozen semen operations of the artificial insemination program are destined to expand to fill needs abroad as well as at home.

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## The Shipping of Semen

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ENOS J. PERRY

In recent years the number of semen shipments sent considerable distances has steadily increased both intranationally and internationally. Today thousands of inseminations are being made daily with shipments of semen transported by bus, rail, and plane.

This has been due chiefly to the growing interest in the artificial breeding of select females to sires considered to be extraordinary. Most of the reported successes have dealt with bull semen, mainly because of the longer time in which it has been available for trial transport. Success has also been achieved in both short and long-distance shipping of the semen of the ram, buffalo, dog and certain other species. In 1957 and 1958, England exported frozen semen to Australia, Ghana, Hong Kong, Iraq, and Kenya with satisfactory results. In like manner, the United States sent shipments to the Pacific area, to most of the countries of South and Central America, and to the Middle East. The lack of dry ice or other comparable refrigerants in some of the importing countries, or inaccessibility to air transport, has caused difficulties at times; but as these are overcome and government health regulations are met, this new type of international business will expand.

### LIQUID SEMEN

Today most of the shipments of liquid semen are within a state or country and seldom involve a period of more than 12 to 48 hours.

The tried method of using a can of solid ice of varying size is usually satisfactory. After the semen has been properly cooled to  $35^{\circ}$  to  $38^{\circ}$  F, the test tube or vial is completely filled to reduce agitation. After stoppering it is sealed by applying melted paraffin with a camel's hair brush. Next it is labeled with the name and number and breed of sire and any other pertinent information, covered with a few thicknesses of wrapping paper, and placed alongside the can of ice. The two are wrapped together lightly in heavy insulating paper and inserted in the shipping container. In the United States this is usually a heavy cardboard box about 8 by 8 by 10 inches of the type used to preserve quart packages of perishables for intervals of several hours. Surrounding the inside are two layers of corrugated cardboard. The refrigerant must be prepared in advance by filling a can with enough water to make a full container of ice after the solid freezing in an electric refrigerator (Figure 89).

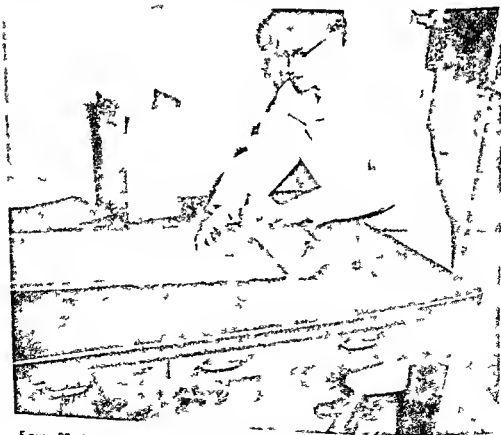


Figure 89 A technician of the New Jersey Cooperative Breeding Association wraps vials of liquid semen in insulated material with a can of solid ice. This type of shipper will hold the semen about 40 hours at  $40^{\circ}$  C.

The size of can to use will depend upon the outside temperature and the period of time that the semen must be held near 40° F. (5° C.). Suitable temperature will be maintained as long as any ice remains. Much care must always be exercised to insure that the shipping container is durable enough to withstand the rugged conditions of transport without damage to the tube (or tubes) of semen. Bhattaeharya (1959) reports that three semen shippers obtained from England, Denmark, and Sweden were tested and tried in India but were not found suitable under the difficult conditions prevailing in India; however, these semen containers are very suitable for the countries in which they were developed. At the time of writing, four containers designed in India and one in Japan are under laboratory test to determine their low-temperature maintaining efficiency. From the work done so far, it appears that two of the containers are comparatively more efficient in maintaining the desirable thermal status under the test conditions of the laboratory.

**Poona Semen Shipper.** This consists of a three-pint Thermos flask held inside a wooden box measuring 8 by 8 by 12½ inches and lined on all four sides, top and bottom, with one-inch thick foam rubber. The maximum loaded weight of this shipper is 12 lb

**Bangalore Semen Shipper.** This is made up of a three-pint Thermos flask held inside a cylindrical aluminum milk pail with lid. The pail is lined on all sides with half-inch-thick foam rubber, and the vacuum flask fits snugly into it. Loaded weight of this container is only about 6 lb.

Sometimes the container is a gallon thermos jug enclosing a pint



Figure 90. Indian and Japanese liquid semen shippers used to distribute buffalo semen.



**Thermos flask** This costs more to use than the lighter shippers but is sometimes believed to be justified because of the comparative certainty of adequate temperature control

After careful sealing and addressing a package should always be labeled "Rush," "Live Sperm," "Perishable," and sent Special Delivery

#### FROZEN SEMEN

The two principal refrigerants used in experimental and routine shipping of frozen semen in recent years are dry ice ( $\text{CO}_2$ ) and liquid nitrogen. Research workers have not yet detected any great difference in the two types of refrigerant as measured by recovered motility and the resulting rate of conception. Each organization or person concerned must decide which of these seems preferable in view of costs and other factors. Dry ice functions at  $-110^\circ\text{F}$  ( $-79^\circ\text{C}$ ) and liquid nitrogen at  $-320^\circ\text{F}$  ( $-196^\circ\text{C}$ ), and hence the latter has a longer safety factor.

**Dry Ice** Ampoules of frozen semen can be shipped in much the same manner as liquid semen when the refrigerant is dry ice. First of all, the container should be one well known for its insulating properties. Suppliers now have on hand equipment that is both strong and light in weight, made of such material as expanded styrene. One of the good shippers is round in shape, has a capacity of 75 ampoules and a loaded weight of 20 lb. Its safety period is three days. Another model especially designed for international transport has a loaded weight of 70 lb, holds up to 500 ampoules, is 18 by 18 by 24 inches in size, and has a safety period of six days. Both models are guaranteed to hold the temperature down to  $-75^\circ\text{C}$  or lower when properly packaged.

As many as a dozen ampoules of frozen semen have also been shipped in a cake of dry ice about 5 by 5 by 9 inches in size. The ampoules are placed either in a deep one inch hole or in several one inch holes drilled into the end of the ice block. The holes are then plugged with chilled cotton and the ice is wrapped in an insulated bag, both are then packaged in a cardboard container as reported by Mixner (1955).

**Liquid Nitrogen** Importing frozen semen with the aid of liquid nitrogen as the refrigerant is being favored in areas where dry ice



Figure 91. Successful arrival of first large shipment of frozen semen from proved United States bulls to Brazil.

is not obtainable, where the nitrogen is available at a reasonable price, and where the electric current is not dependable. One manufacturing company in the United States has been making a container in various sizes that is refrigerated by the liquid nitrogen, and this can be used for both shipping and storing. This outfit has a vertical storage system. The ampoules are fastened end to end to metal strips known as "racks." One of these combination shippers and refrigerators has a capacity of 504 ampoules, and the largest size accommodates over 40,000 ampoules. The racks are packed in six cylindrical metal canisters measuring  $2\frac{3}{4}$  inches in diameter and  $14\frac{1}{4}$  inches in height, and each canister holds 84 ampoules. The American Breeders Service at Chicago, Illinois, and Madison, Wisconsin (1957), assisted in the development of this equipment. The particulars of the one with a capacity of 504 ampoules are as follows:

Height over all	22 in
Outside diameter	17½ in
Weight empty	62 lb
Semen capacity (packed in canisters)	504 ampoules, 14 lb
Liquid nitrogen capacity when loaded with semen	27 liters 48 lb
Total weight when fully loaded with semen and liquid nitrogen	124 lb
Temperature maintained until exhaustion of liquid nitrogen	-196° C (-320° F)
Temperature holding time (dunning field use without refilling) approximately	3 weeks
Daily consumption of liquid nitrogen approximately	1½ liters
Cost of 27 liters of liquid nitrogen	\$5 00 (U S)
Cost of refrigerator and full complement of accessories approximately	\$546 44 (1958)

The temperature holding time of the largest of these shipper refrigerators is two months. Hence, it can be loaded to its capacity of 42,900 ampoules and carried by ship from one country to another. Its loaded weight is 2316 lb. The liquid nitrogen capacity is 484 liters (826 lb), and the daily nitrogen consumption is approximately 7 liters. Its height with legs is 4½ feet, and the outside diameter is 4 feet. The cost was about \$9800 fully loaded, in 1958.

The names and addresses of companies that sell any of the above-mentioned equipment, including those that offer machinery for the making of dry ice and liquid nitrogen, can be obtained by communicating with one of the agricultural experiment stations or with one of the large artificial breeding organizations that has been in operation for a period of several years.

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## Disease and Artificial Insemination

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and  
LESTER L. LARSON

Artificial insemination is providing numerous advantages toward the improvement of livestock and poultry in many areas of the world. Although the technique is practiced most widely in cattle, its success in sheep and poultry is impressive. Interest in insemination of swine is growing actively, and insemination of horses and dogs is practiced successfully on a limited scale.

Particularly in cattle, the technique has provided not only a means of inexpensive, mass genetic improvement but also a critically needed method for the improvement of fertility. Positive, effective control of venereally transmitted diseases affecting reproduction becomes possible when the relatively few males required in artificial insemination are maintained under continuous technical supervision. Further, it is possible to employ, selectively, the semen of bulls of established fertility. In some countries, where long-practiced "communal" breeding practices had made infectious reproductive diseases ubiquitous and crippling to the cattle raising economy, fertility improvement through control of disease has been the primary motivation in the extension of artificial insemination.

In other varieties of livestock and poultry, control of infectious

diseases affecting fertility is of somewhat less importance because the diseases are apparently not only less genital-specific and less numerous but also less common. For these animal species the principal hygienic advantage of artificial insemination may be that it eliminates the necessity of frequent introduction of males of uncertain general health status from outside herds or flocks.

Artificial insemination has a unique advantage in stopping sexual contacts. There need be no males carrying venereal infections from female to female. The male becomes part of a laboratory and mechanical process which can be brought under rigid technical control. This process substitutes for natural service, wherein only limited or no human supervision or restraint is exercised.

Artificial insemination makes possible the fertilization of many thousands of females per year from a single male. One bull, maintained under rigid technical supervision, can replace hundreds of unsupervised bulls in natural service. Their thousands of promiscuous sexual contacts are eliminated.

Today, artificial insemination of cattle has advanced, and it must now stand realistic and critical judgment as to its true usefulness. It is a 'sword with two edges.' It has been and continues to be our most effective weapon in the control and eradication of certain diseases. But whenever it is operated in an irresponsible, negligent, incompetent, or ignorant manner, it has the potential of spreading infection from diseased males further and faster than natural routes of disease transmission. Also, an inseminating technician does travel from herd to herd. He must consistently perform in a responsible manner and observe the principles of sound farm sanitation. Although the over all record of artificial insemination has been good, unfortunate incidents have occurred, and they have been of serious consequence. There exists a very real responsibility that must never be taken lightly.

As to disease, artificial insemination is advantageous to a livestock population only when all semen originates from sources under technically competent supervision and responsible management and when the semen is administered by well trained, inseminating technicians functioning in a responsible, sanitary manner. Breeding by artificial insemination can eliminate the spread of numerous diseases, both systemic and sexual, which are often transmitted in

natural mating. Also, it can block the route by which venereal infections are transmitted from one individual to another.

#### DISEASES IN CATTLE

**Bovine Venereal Trichomoniasis.** This is a venereal disease of cattle caused by a protozoan parasite, *Trichomonas foetus*. Natural transmission is almost exclusively by sexual contact. Infected bulls ordinarily exhibit no clinical symptoms, but evidence appears in the health records of the cows to which they are bred. Once infected, bulls usually remain permanently infected and will transmit their disease to a high percentage of susceptible females on sexual contact. Bulls can be cured of the infection by specific treatment.

This infection is self-eliminating in females, but this may take six months or longer. During the period of infection there is a state of infertility. For a few years thereafter, immunity to reinfection and consequent normal reproduction from services by an infected

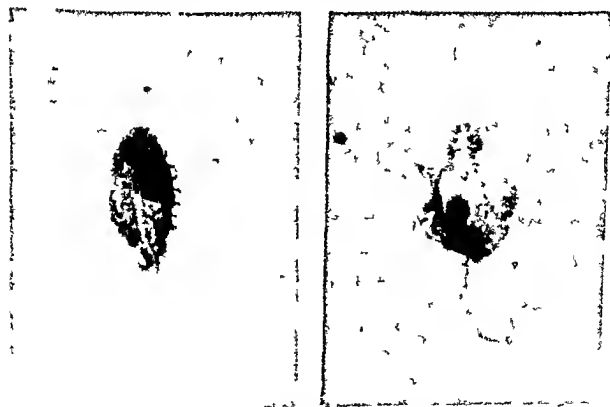


Figure 92 Two specimens of *Trichomonas foetus* ( $\times 1600$ ). This protozoan organism has three anterior flagella and an undulating membrane terminating in a posterior flagellum.

bull are usual; then, however, re-exposure may result in reinfection. In females, the course of the infection and the period of infertility are not ordinarily altered through treatment.

Trichomonads cause inflammation of the interior of the uterus. Any developing calf dies, but usually so early that nothing is noticed except that the cow returns to heat three to five weeks after breeding. Rarely, a pregnancy progresses for two or three months before it is interrupted by the infection. Then, abortion of a fetus may be seen. Rarely, after the death of the developing calf, pus may accumulate in the uterus in quantities up to several gallons. A cow so affected may require special treatment to restore the uterus to health (Bartlett, 1949).

The effect of bovine venereal trichomoniasis upon the reproductive efficiency of a herd of cattle allowed to mate naturally is serious (see Table XXXI) (Bartlett, 1947). Surely, the acceptance and

TABLE XXXI TEN YEAR SUMMARY OF REPRODUCTION IN HERD A \*

	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946
Av ♀ ♀ per month in breeding herd	67	66	68	72	74	77	66	62	61	61
No susceptible ♀ ♀ added during year	7	9	14	12	11	23	15	8	12	13
No ♀ ♀ dropped during year	5	9	9	8	15	15	30	13	9	7
No services or inseminations	104	97	109	117	130	204	206	137	103	93
No recognizable pregnancies initiated	60	53	59	57	37	51	38	47	54	52
No calves	66	53	55	59	58	36	36	44	56	55
Av lax in days per pregnancy from 1st coitus to initiation of a recognizable pregnancy or last coitus prior to disposal	28	28	33	39	72	121	141	38	28	29
Av of percentage of days of normal reproduction per month	90	86	89	87	76	57	51	78	88	87

Source Bartlett, 1947

\* Bovine venereal trichomoniasis probably introduced June 1941. Bovine venereal trichomoniasis diagnosed September 1943.

growth of artificial insemination has had a large part in the reduction of bovine venereal trichomoniasis in the dairy cattle populations of several countries.

Recently, bovine venereal trichomoniasis has been reported to be far more common in beef cattle under range conditions in the United States than previously recognized. Three hundred and eighty-three bulls in eight areas were studied. Infected bulls were found in half the areas, the incidence of trichomoniasis being from 5 per cent to 18 per cent in those bulls present. These infected bulls had been

used either on public range or for breeding heifers later turned out on range (Fitzgerald *et al*, 1958)

Artificial insemination with semen from disease free bulls provides the most certain, quickest, most practical, and economical means of controlling bovine venereal trichomoniasis in a group of females

Repeated laboratory tests must be made to ascertain that bulls used in artificial insemination are free from *Trichomonas foetus*, as this organism survives the conditions of semen processing—including freezing

**Bovine Genital Vibriosis.** This is a venereal infection of cattle caused by a bacterium, *Vibrio fetus*. This disease is probably far more widespread than trichomoniasis, which it resembles in certain respects. In fact, differential diagnosis often requires careful laboratory studies. Bulls are the permanent reservoirs of infection. Females eventually recover and regain their abilities to reproduce after variable periods of infertility that are associated with infection of the uterus (McEntee *et al*, 1954)

The frequency of vibriosis is high in the dairy herds of some areas. In some groups of bulls assembled for artificial insemination purposes, the incidence of infection upon intensive culture studies has been revealed to be as high as 50 per cent (Hughes, 1956). At present, *Vibrio fetus* is usually controlled in artificial insemination procedures through the addition of antibiotics to the semen. It has been shown by Orthey and Gilman (1954) that a combination of 500  $\mu$ g of streptomycin and 500 units of penicillin per ml of diluted semen inactivates *Vibrio fetus* provided the rate of dilution is at least 1:25 and the elapsed time before use of the semen is at least six hours.

Vibriosis has required special consideration in processing of frozen semen because it was discovered that glycerin interferes with the activity of the antibiotics usually employed in semen processing. Diagnosis and treatment of infected bulls and revised methods of semen processing are essential, new procedures.

The technical problem of diagnosis of vibriosis in either bulls or cows and the difficulty of restricting contact and transmission between the sexes under the practical conditions of the barnyard and range are formidable. Properly conducted artificial insemination provides, today, the only solution to the problem of vibriosis in an infected herd.



**Bovine Brucellosis.** Transmission of bovine brucellosis, or Bang's disease, is usually by oral ingestion of organisms shed in the genital discharges of an infected female. Transmission to females by sexual contact, even though an infected "semen shedder" bull is involved, is exceedingly rare or may not occur at all. However, when semen infected with *Brucella abortus* is placed through the cervix as is usual in artificial insemination, transmission of brucellosis has occurred at a very high rate. Special efforts must be made to provide necessary safeguards against use of infected bulls in artificial insemination by careful and repeated testing of bulls for brucellosis. Both serum agglutination and semen plasma agglutination methods are employed.

**Bovine Tuberculosis.** This infection may affect the genital organs of cattle, and venereal transmission has been recognized. The theoretical possibility of transmission by artificial insemination must be given due consideration through periodic testing of bulls to re establish their freedom from tuberculosis.

**Leptospirosis.** Transmission of this systemic disease of cattle by artificial insemination has not been demonstrated. However, a theoretical basis for transmission exists. Shedding of the causative organisms from the kidney by way of the urine is a common characteristic of this disease. Survival of *Leptospira pomona* in semen processed with antibiotics and held for one week at 38° F. has been demonstrated. Transmission of the disease through application of contaminated urine upon the skin of the legs of susceptible cattle has been shown. When these facts are taken together, the assumption seems reasonable that transmission could occur through deposition of infectious semen in the uterus of susceptible females.

It is essential that only bulls free from leptospirosis be used in artificial insemination.

**Pustular Vaginitis (Coital Exanthema).** This condition, though common in some parts of the world, is very rare in the United States. Its primary symptoms are pustular ulcers of the vagina. Sometimes the preputial membranes of bulls are observed to be affected with similar lesions. Although the primary route of transmission of this infection apparently is not venereal, it seems reasonable that it could spread by this route and that artificial insemination could also serve as a means of transmission. Thorough knowledge of the health status

of bulls and the herds from which they originate provides the most reasonable safeguard against this infection.

**Foot and Mouth Disease (Aftosa).** The possibility exists that semen might be contaminated with the virus of this disease. The virus has been shown to survive the conditions of semen processing and freezing. Consequently, transmission of this infection from endemic areas exists as a potential danger.

**Granular or Nodular Vaginitis.** The exact etiology of this condition has not been established. Artificial insemination does have the advantage of mechanical placement of semen beyond the area of affection. This condition is not considered to be of major importance in artificial insemination.

**Miscellaneous Infections.** Certain rare sexual affections of cattle have been reported from limited areas. These have not been sufficiently well studied to establish their relationships to or importance in artificial insemination. Epivaginitis is a venereal disease of cattle described in Africa which is destructive to the epididymides of males and to the uterus and Fallopian tubes of females. From England, a viral infection of the vagina and uterus characterized by mucopurulent genital exudate, uterine edema, abortion, and infertility has been described. A somewhat similar infection has recently been described in California. A type of endemic abortion of cattle of unestablished etiology characterized by a peculiar liver lesion of the abort has been described in California.

#### HEALTH STANDARDS FOR BULLS IN ARTIFICIAL INSEMINATION

Minimum standards for health of bulls used in artificial insemination of cattle have been recommended by the Special Committee on Animal Reproduction and Artificial Insemination (1956) of the American Veterinary Medical Association to the National Association of Artificial Breeders (Bartlett *et al.*, 1957). These recommendations, adopted by the N.A.A.B. for guidance of their membership, are as follows:

**General Sanitary Practices.** The continuous guidance of a veterinarian who is encouraged to become thoroughly familiar with the health problems and routines can be highly constructive in maintaining the security of individual artificial insemination organizations. Problems of a special nature concerned with general sanitation, hygiene, and disease control,

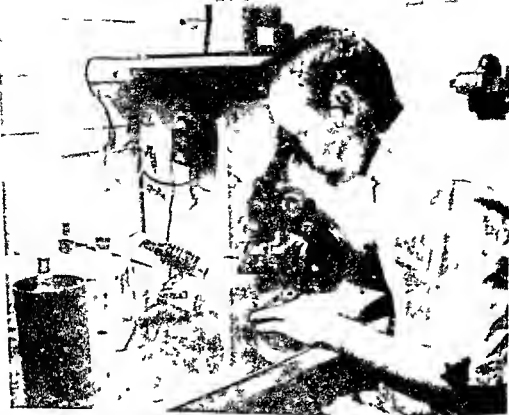


Figure 93 Examining a sample of semen for pathogenic organisms, of the South eastern Pennsylvan a Art ficial Breeding Cooperative, Lancaster

not included in the specific recommendations, should be handled in accordance with the best of modern scientific principles

Care should be constantly exercised to prevent the introduction of infectious diseases onto stud premises. Routines should be scrupulously designed to prevent transmission of disease from bull to bull. Artificial vaginas should be thoroughly disinfected after use or, ideally, a separate set of equipment assigned to each bull. Pipettes, douching apparatus, and all other equipment which touch the genitalia should never be used in common without disinfection, disease has been inadvertently spread by such means.

Accidental natural service of mount cows, followed by another subsequent accidental service by another bull, has serious potential for intra-stud transmission of both bovine venereal trichomoniasis and bovine vibriosis. Accidental services should be guarded against and prevented to the greatest possible degree. Bulls or steers should be the preferred mounts whenever possible.

Scrupulous care should be exercised by the collector to assure that his hands cannot serve as a means of transmission of infectious material from bull to bull. At least, the collector's hands should be thoroughly washed and dried between collections from different bulls. Some operators may choose to wear rubber gloves, using a different sterilized pair for each bull.

**Herds of Origin.** Knowledge of the detailed health status of each new bull, including health status of his herd of origin, is fundamental in the protection of health security of the entire stud, as well as for the economic protection of the owner in whose herd the semen is to be used. Isolation and observation of new bulls are desirable.

**Bovine Tuberculosis.** Bulls should be found negative upon test for tuberculosis before being admitted to a stud for use in artificial insemination. Thereafter, tests should be made not less often than annually for as long as a bull remains in artificial insemination.

**Bovine Brucellosis.** Bulls should be found negative to a blood serum-agglutination test, meeting the requirements of the state in which the bull is to be located, before being admitted to a stud for use in artificial insemination. In addition, each bull should be found negative to a second blood test and to a semen-plasma agglutination test (1:25 dilution, tube method) before semen is released for use. Thereafter, blood and semen-plasma tests should be made not less often than each six months for as long as a bull remains in artificial insemination.

**Bovine Venereal Trichomoniasis.** It is advisable that the herd of origin of each new bull be investigated for possible presence of this infection before his acceptance. Bulls should be found negative at several examinations before semen is released for use.

Upon admittance to a stud, a series of six negative examinations, each conducted at intervals of not less than one week, should be carried out. Thereafter, every bull should be examined not less often than each six months for as long as a bull remains in artificial insemination.

One examination shall consist of detailed, direct microscopic study and, preferably, also, protozoological culture of a single collection of semen obtained and handled in an acceptable manner.

**Bovine Vibriosis.** Presently, dependence upon addition of antibiotics to semen extender is essential for vibriosis prophylaxis; this method has been demonstrated as highly effective. Semen extender should contain at least 500 micrograms of streptomycin and 500 units of penicillin and be so treated for at least six hours before use, in accordance with the recommendations of Orthey and Gilman.

**Bovine Leptospirosis.** Bulls should be found negative to a blood test for leptospirosis before admittance to a stud for use in artificial insemination.

Thereafter a test should be made not less often than each six months for as long as a hull remains in artificial insemination. Unfortunately, there is not yet available a standard official test for leptospirosis. It is recommended that the testing facilities in the state concerned be utilized and that interpretation be based upon the judgment of the laboratory responsible. Bacteriological examination of semen from low level reacting bulls may be found useful in arriving at sound decisions.

#### DISEASES IN SHEEP

**Ovine Balanoposthitis** Ovine balanoposthitis is the venereal manifestation of the virus complex known as ulcerative dermatosis of sheep. Lip and leg ulceration is associated with the same virus. In this disease lesions around the prepuce are first vesicular. Later, they coalesce, form ulcers and eventually scabs. Healing is slow, and without treatment the lesion may remain active for prolonged periods of time. In the ewe lesions start near the lower portion of the vulva and spread in some instances over the entire vulva. The vagina usually is not involved. The disease is usually self limiting in the female; acute symptoms persisting not more than ten days.

Transmission of the disease is almost exclusively by venereal contact. A flock outbreak results when an infected ram is placed with susceptible ewes. Rams should be examined for preputial lesions prior to being placed with a flock and those showing lesions should be isolated and not used. In a severe flock outbreak it may be necessary to stop all breeding operations. The use of artificial insemination with disease free rams could make it possible to resume breeding earlier.

Artificial insemination can provide an effective means for control of this infection. But likewise it is necessary to be sure that rams used in artificial insemination are free from the infection.

**Ovine Vibriosis** This disease caused by *Vibrio fetus*, is the most frequent cause of enzootic abortion in sheep in the United States. In an infected flock up to 10 to 20 per cent of pregnant ewes may abort. Usually abortion is the only sign of disease in the ewe. However, sometimes metritis and fetal membrane retention occur, and these may account for a death loss of up to 10 per cent of ewes that abort. Vibriosis of sheep is spread primarily through ingestion of contaminated genital exudate. Most research indicates that the disease is not venereal in nature and by implication that artificial



Figure 94. Veterinarian collects specimen from prepuce of bull to test for bovine venereal trichomoniasis. (Courtesy American Breeders Service, Chicago)

insemination would not play an important role either in control or transmission of this disease.

**Ovine Enzootic Abortion.** This disease is caused by a rickettsia-like organism and results in abortion, retention of fetal membranes, and severe metritis. It has been reported in Scotland, England, New Zealand, Germany, and Italy. The possible relationship to artificial insemination has not yet been established.

**Epididymitis in Rams.** This disease, first studied thoroughly in New Zealand, has recently been reported in California. It is characterized by a chronic inflammation of the epididymis and results in fibrosis and ultimate sterility if both epididymides are involved. The cause is an organism which somewhat resembles *Brucella*. Artificial insemination in which non-infected rams are used should have value in control of this disease.

#### DISEASES IN SWINE

**Swine Brucellosis.** This is an insidious disease of swine that often is transmitted venereally. Abortion and infertility are the common

symptoms in an infected herd. The boar, in contradistinction to the bull, is very susceptible to *Brucella suis* infection either by genital contact or other routes. When the boar is infected, the organisms frequently localize in the testicles and accessory sex glands. These organs serve as a focus of infection and the organisms are shed by way of the semen. Infected semen is a common source of exposure for the sows and gilts.

Recent progress made in artificial insemination of swine strongly suggests that artificial insemination will play an important role in the future of the swine industry. Since brucellosis of swine is largely found in purebred herds and the boar plays such an important role in the disease's perpetuation and spread, it would appear that use of semen known to be from disease free boars would be very effective in breaking the cycle of this important venereal disease.

**Leptospirosis.** In swine, this disease in its essential characteristics and manner of transmission is very similar to leptospirosis of cattle. It is probable that this disease could be spread by artificial insemination should infected boars be employed. On the other hand, however, artificial insemination using boars which are known to be *Leptospira* free could be of considerable value in the control of this infection.

#### DISEASES IN HORSES

**Dourine.** This is the only trypanosome disease known to be transmitted venereally. The causative organism is *Trypanosoma equiperdum*. In the past this infection has been present both in the United States and in Europe. Presently, the infection is apparently nonexistent in the United States.

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## Feeding and Management of Sires

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JOHN W. BARTLETT

The feeding and management of sires has always been an important factor in obtaining high conception rates in our herds and flocks. Those sires that are to be used in artificial insemination require even greater care than those used for natural mating. The proved sire is of course in the prime of his natural life before his true worth is well established. To maintain him in a healthy, vigorous condition for a long time is most essential.

### THE YOUNG BULL

The young sire must be brought to the full development of his physical inheritance if his type, size, and breeding powers are to be definitely known. A poorly fed and badly managed young sire may be such a disappointment that he will not be used, on the other hand some valuable germ plasma may be discarded because he has not been allowed to develop.

The requirements for growth are very different from those for the maintenance of the body. A young, growing sire suffers sooner and much more seriously from nutritive deficiencies than does an old sire. A much larger amount of total digestible nutrients must be provided for the growing sire than for one which merely needs to remain at constant weight. What the young sire can use for growth

is that which is left after his body has received enough for maintenance. He should not be maintained on roughage alone, since he needs ample grain for the development of tissues and muscles. For the development of the skeleton large amounts of minerals are necessary, especially calcium and phosphorus. Vitamins are required in greater proportion for the young sire than for the animal which merely needs maintenance. Rations which supply A and D are sufficient for the young four-footed farm stock. Poultry, however, require large amounts of G. The rates of growth of young sires vary with the species and also within certain breeds of the same species.

Young bulls should be started on whole milk and allowed up to one-tenth of their weight for the first 30 days, and should be continued on skim milk or a good milk substitute for at least six months. Recently it has been found advantageous to supplement the ration with vitamins A and D for rapid growth and development. Vitamin B<sub>12</sub> is used by several well-known breeders, especially during the first month, because it is at this time that the young calf needs to get a good start. Most breeders recognize that calves will relish a mixed hay and will begin to nibble on a small quantity when a few days old. They should be allowed free access to it at all times. A good grain ration of equal parts of cracked corn and crushed oats is excellent for the young bull calf. Some prefer to feed the same ration as is fed to the older herd. Where grass silage is available, it seems to fit well into the calf's diet, and it is also a source of vitamin A.

When six months of age, a young bull should receive from 4 to 6 lb. of grain daily; and if good pasture is available, he may be turned out. Hay, however, should be placed in a rack in the pasture lot. It will be found a very great aid to growth, and young animals will eat liberally of it. If well-grown, the young bull is ready for light service when twelve months old, but he should not be used more often than once a week. At this time a ring should be put in his nose, and he should be taught to lead; because for the rest of his life he will be handled regularly on a lead strap or staff.

One excellent method of handling a young bull is to put a chain around his horns, clamp it together in the middle of the forehead, and bring it double down his face and through his ring. The two ends of the chain can then be fastened to a second ring which will hang three to four inches below the one in his nose (Figure 96). This method teaches him while young that he is to be led, and

he can always be easily caught. As the bull grows older a heavier chain will have to be substituted.

Since it is the plan of most artificial breeding associations to use proved sires for the most part, a problem arises as to where the young, unproved sire may be kept. It is suggested that after the yearling bull's semen has been used to breed 500 to 1,000 cows he then be retired until proved. It is unwise to place the young bull on a private farm while waiting for a proof on his daughters. The future use of such a sire may be ruined as a result of natural service in a herd where some infection may exist. Most associations provide quarters in the hull stud while the young bull is being proved. In emergencies he can be used, and also there may be some breeders who will wish to have his semen in their breeding program.

The association is the place to try out young males, and no one member will have more than one or two daughters, should the sire fail to prove meritorious. As has been said, it is much safer to have proof data from several farms than from one herd.

#### THE BULL IN SERVICE

When a sire comes into use, his management should be so planned that he may remain in service for several years. The best housing for him is a box stall with a swinging door which will allow him freedom to go out to his own paddock for exercise and sunshine. If sires can be quartered in adjoining box stalls and paddocks where they are always in sight of one another, there is a psychic factor which increases their service activity.

Bulls vary greatly in their capacity to produce semen regularly and of high quality. Where liquid semen is to be made available at all times, bulls are being used for semen collection at intervals of three to four days. Glycerol is added to the semen, keeping it viable for four and five days and the conception rate is well maintained. In some cases it is necessary to train the sire to ejaculate at three- to four-day intervals. Reports indicate that this can be done with most bulls, although it may take a few weeks to get the sire into a regular routine. Sires that have become injured in hips or legs or have become too lame to mount for collection can also be trained to ejaculate when a teaser cow is brought near them.

If frozen semen is used in the program of an association, collection on a weekly basis can be the general rule. The manager of the stud

should learn the possibilities of each sire and rest him accordingly. When bringing a new sire into service involves hauling or shipping, it is recommended that he be rested ten days to two weeks before the first semen sample is collected.

The best service bull is generally the one which is kept in a thrifty condition, but not fat. A good mixed hay for roughage is advisable, and a liberal amount of grass silage seems to aid in the production of viable sperm. The amount of roughage can best be determined by the condition of the bull. He should not have enough to allow him to get paunchy. When hay is the only roughage given, a daily allowance of 15 to 25 lb. should be sufficient. Green soiling crops are an excellent conditioner if the sire does not have access to pasture. Bulls, like cows, need ample water, but they should not be watered just prior to service or they may be slow and sluggish.

If good roughage is fed, 6 to 8 lb. of concentrates daily should be ample to keep most bulls in the desired condition. Although the protein content of the ration will depend on the quality of the



Figure 95. Hoof trimming is not a difficult job when the right tools are used. Here a wood chisel is used to remove the horny growth of the hoof. Tranquilizers may be given to quiet the bull for this operation but should not be given to the stallion.

roughage, it is generally a good policy to use a conditioning ration containing about 12 per cent total protein. Some bulls need exercise if they are to remain in good service. Turning two or more into a large field is a practice that may be followed if the horns have been removed. Some breeders fix a cable between two posts or two buildings, the bull is then tied to a swivel on a pulley which permits him to move around without becoming tangled up in his own lead chain or rope. Electric exercisers can be made very easily by a bandyman if he can secure the rear end of an old car or truck and build a sweep around it (Figure 96). The rear is stood on end and geared to a small motor. Arms radiating at equal intervals from the axle, so spaced that no two animals can get together when the sweep is in motion, lead the bulls in a circular path. A half hour's walk each day on such an exerciser also helps to keep the bull tractable, and is especially beneficial in keeping the feet and legs in condition. Foot trimming is often a must with mature bulls. A box made of plank may be used as a foot rest, and an ordinary wood chisel is handy in cutting away excess growth of the hoof (Figure 95). Bulls with long toes often become lame, and their usefulness is impaired.

Numerous ways have been devised for managing the bull when collecting the semen. Some prefer to place the cow in a breeding rack and lead the bull out of his pen by a lead strap or bull staff. Others have made a ceiling track to which the lead chain is attached on a swiveled pulley, and the bull is thus led to the cow or dummy, he is less dangerous, as he cannot get his head down. A third method is to lead the bull along a fence, the leader walking on the opposite side or along a raised walk so that the animal has no chance to attack him.

In one New Jersey bull barn the cow is taken to the bull's pen, and the collection is made there. If a double rope from the stanchion to a ceiling pulley at the rear of the pen is used, the bull can be pulled over to the rear wall while the cow is led into the pen and stanchioned. If a rope which can be snapped into the bull ring is used, the bull can be led from front to rear of his pen at the will of the person in control of the ropes. Releasing the rope gives the bull enough freedom of his head to mount the cow and serve the artificial vagina. Immediately afterward he can be pulled to the rear wall, and the cow can be taken out of the pen.

The lead rope will then permit bringing the bull back to his stanchion, from which point the ropes may be unsnapped. Experience has shown that many bulls do not lead well or serve well when moved on a staff. The psychology of bringing the cow to the bull's pen has sometimes caused a bull to yield a larger sample of semen.

#### THE STALLION

One of the most important phases of stallion management is exercise. Nothing is more vital to his breeding ability than that he be given some work to do. A half-day's work in the harness is recommended. Since mares are usually bred to foal in the spring, the stallion will commonly experience the demand for semen at one season of the year. He should, therefore, be built up for this period and maintained by proper management. His diet should contain first-class roughage with ample amounts of protein, mineral matter, and vitamins. The best roughage is a mixture of legume and bright green timothy hay. It should by all means be free from dust, and the amount should not be in excess of what he actually needs.

The nature of the concentrates for the stallion will depend upon the type of hay. With timothy hay, a good ration is oats (4 parts by weight) and bran (1 part); or oats (4 parts), corn (6 parts), and bran (3 parts). Mature stallions can be fed oats as the only concentrate. When the roughage is largely legume, most horsemen prefer not to include more than one-half corn in the grain mixture for stallions. No specific amount of grain can be prescribed, since this depends on the amount of exercise given and whether the horse is a hard or easy keeper. Most breeders prefer to have the stallion thrifty at the beginning of the mating season and to see him gain a little weight as the season progresses.

Grooming and care of the feet are essential, and regular attention should be given to how the horse walks. Drugs and stock tonics are not necessary to a vigorous stallion.

#### THE RAM

The ram can be kept in a healthy, thrifty condition on roughage alone, except during the breeding season. Bright legume or non-legume hay accompanied by a light feeding of silage is satisfactory.

During the summer good pasture alone is sufficient. At least one month prior to the breeding season the ram should be conditioned by grain feeding. During heavy service he should be fed 1 to 2 lb. daily of a grain mixture containing about 14 per cent total protein.

A good mature ram will serve 25 to 50 ewes when he runs with the flock all the time, or nearly twice that number when he is turned in with the flock only a few hours each day during the breeding season. When used artificially, he can be inseminated to many hundred ewes, if they are available, without heavy or different fodder.

#### THE BOAR

Overfeeding and a lack of exercise are common causes of unsatisfactory service in boars. A very thin, run-down condition should also be avoided.

During the summer, boars on pasture will get plenty of exercise. In the winter they can be sheltered at one end of a long paddock and fed at the other end to encourage exercise.

Except during the breeding season a boar should not be fed more than 1 to 1.5 lb. of grain daily per 100 lb. of body weight. Then the ration should be increased somewhat two weeks before the breeding season starts so that the boar will gain weight at that time.

A boar is old enough for very light service at eight months with 15 collections per season. A mature boar should not be required to deliver more than two collections per week or three per fortnight, over any considerable period.

#### MALE FOWL

During the heavy breeding season the male bird should be given careful attention. It is important that he get sufficient feed to maintain himself in vigorous, thrifty condition. It is advisable to allow male birds free access to a 20 per cent protein breeding mash fortified with sufficient vitamins A, B, and D. A limited grain ration mixture composed of 2 parts corn, 1 part wheat, and 1 part oats should be added. Other grain mixtures may be equally satisfactory. It is necessary to limit the grain mixture to make sure the male bird eats sufficient breeding mash. A cage 4 feet by 4 feet should provide sufficient space for exercise.

## MALE TURKEY

Male turkeys should be kept in good condition during the breeding season, but not too fat. Rations and breeding practices similar to those already suggested for male fowl should be satisfactory for turkey toms. Males need plenty of exercise and should be kept in individual yards approximately 20 feet by 20 feet to avoid injury that may result from fighting. If cages are used, the floors should be solid.

Under flock conditions, turkey growers often have difficulty in maintaining satisfactory egg fertility. They resort to the use of individual stud pens, or make a practice of rotating groups of males at given intervals in order to encourage proper mating. They also find it necessary to remove the toe nails or blunt the spurs by sawing or filing to avoid back injury to the hens. A canvas saddle may also be placed on the back of the hen to prevent tears and other injuries. Females with severely injured backs tend to produce infertile eggs. Artificial insemination offers a possible solution to these problems of management.

## MANAGING THE SIRE AT SERVICE TIME

Certain male farm animals are sensitive to artificial collections, whereas others seem able to endure considerable rough handling. Intelligent management at service time helps to keep them keen, and hence is an aid in obtaining the normal quantity of good semen. Some practical facts pertaining to the sexual nervous system of the bull are now available. Part of the information gained is also applicable to certain sires of other species.

The well-known reflexes, such as mounting the cow, projecting the penis, thrusting and ejaculating, can easily be retarded or even inhibited in a bull by unnatural methods of handling him. The basis of his natural behavior at time of semen collection or natural breeding is the gratification of the physical sense. The majority of bulls will respond most satisfactorily if they are permitted to serve each time in familiar surroundings and are handled by the same attendant, provided these are associated with previous satisfactory experience.

Hammond *et al.* (1947) expertly treat the subject of sexual reflexes as follows: "The sexual reflexes are inhibited if their perform-



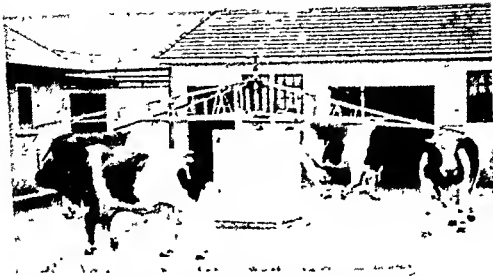


Figure 96 Bull exerciser at the Dairy Research Farm of the New Jersey Agricultural Experiment Station, Sussex. By means of the chain and ring construction on the bulls' heads they may be effectively caught and controlled. For leading, a staff should be snapped to the nose ring.

ance is accompanied, not by pleasurable sensations, but by sensations which are painful, uncomfortable, or even distracting. In very sensitive bulls inhibitions may arise quite quickly, even when the collections are taken most carefully. Repeated careless collections will 'put off' the most hardy bulls. Inhibitions will be reinforced the more often the unfavorable conditions are repeated, and the shorter the interval between collections. If inhibition has started, it is a great mistake to persist with unsuccessful attempts at collection. The animal should be given a rest from collections for as long as possible. Inhibitions are also reinforced if repeated in familiar surroundings. The sight of the artificial vagina or the operator and the color of the teaser cow can be factors. In these circumstances slight inhibitions which have developed may sometimes be overcome by changing the surroundings as much as possible."

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## Notes on the Contributors

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The men whose contributions make up the third edition of *Artificial Insemination of Farm Animals* have conducted research and practical programs in artificial breeding on three continents.

The two British contributors, Dr. Joseph Edwards and Dr. T. Mann, provide both fundamental and highly specialized information on the reproductive physiology of farm animals. Dr. Edwards, author of the chapter on the organs of reproduction, is chairman of the Production Division of the Milk Marketing Board of England and Wales. He holds the M.Sc. degree from both the University of Cambridge and the University of Minnesota, and the D.Sc. from the University of Glasgow. He is president of the British Society of Animal Production and a governor of the Royal Veterinary College.

Dr. Mann, who wrote the new chapter on the evaluation of semen by chemical analysis, is director of the A.R.C. Unit of Reproductive Physiology and Biochemistry at the University of Cambridge. His book, *The Biochemistry of Semen*, was published in 1954 and remains the standard text on the subject. He holds the M.D., Sc.D., and Ph.D. degrees and is a Fellow of the Royal Society.

The new chapter on the use of frozen semen, one of the newest techniques in artificial breeding, was contributed by H. A. Herman, executive secretary of the National Association of Artificial Breeders of the United States. Dr. Herman is editor of the *A.I. Digest* and co-author of two books, *Artificial Insemination of Dairy Cattle* (with F. W. Madden) and *Dairying Laboratory Manual and Outline* (with A. C. Ragsdale). He holds the Ph.D. from the University of Missouri, where he was formerly professor of dairy husbandry. Supplementing Dr. Herman's chapter is the revised chapter on the shipping of semen by the editor, Enos J. Perry.

Dr Ralph P Reece, contributor of the chapter on the role of hormones in reproduction, is professor of dairy husbandry at the College of Agriculture of Rutgers University, and research specialist at the New Jersey Agricultural Experiment Station. He has served on the Biological Research Committee of the Planned Parenthood Federation of America. Dr Reece wrote the chapter on mammary gland development and function in *The Endocrinology of Reproduction*, edited by J T Velardo, published in 1958.

From Norway comes the new chapter on swine, written by John Aamdal out of his wide and successful experience as a veterinarian on the staff of the Royal Veterinary College at Oslo. In Norway, artificial breeding of swine has so increased in importance as to be second to the cattle programs.

Dr P Bhattacharya, author of the new chapter on buffaloes, is assistant director and head of the Division of Animal Genetics of the Indian Veterinary Research Institute at Itaznagar. He holds the Ph.D. degree, and among his professional connections are memberships in honorary societies in Italy and England as well as his native India. He is official correspondent in India of the Commonwealth Agricultural Bureaux for Animal Genetics and Breeding and has contributed chapters to textbooks and practical handbooks of animal husbandry.

The chapter on dogs has been rewritten to include the most recent developments in the field by its original author, Dr Ellis P Leonard, head of the Department of Therapeutics and Small Animal Diseases at the Cornell University College of Veterinary Medicine. Dr Leonard is co-author of *Canine Medicine*.

Enormous strides have been made in the artificial breeding of chickens, turkeys, ducks, and geese since Professor F P Jeffrey prepared his chapter for earlier editions of this book. He has been assisted in this revision by his colleague at the University of Massachusetts, Dr J Robert Smyth, Jr. Dr Smyth is research professor of poultry husbandry at the College of Agriculture. Professor Jeffrey is associate dean of the college and co-author (with D R Marble) of *Commercial Poultry Production*.

Dr Victor Berliner, formerly associate professor of animal husbandry at Mississippi State College, and now director of the Division of Endocrinology at the Ortho Research Foundation, Raritan, New Jersey, has expanded his original chapter on horses and jackstock. He is a contributor to *Reproduction in Domestic Animals*, edited by H H Cole and P T Cupps, published in 1959.

At the time Dr Clair E Terrill wrote the original chapter on sheep

Since 1955, when Professor Perry retired from Rutgers, he has served the United States government through the International Cooperation Administration in Egypt, as Animal Husbandry Advisor (1956), in Lebanon, as Dairy Advisor (1957-1958), and in Brazil, as Livestock Advisor (1959) Professor Perry conducted a 1958 world survey of artificial breeding for the National Association of Artificial Breeders

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